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HISTOCHEMICAL STUDIES ON ACID AND ALKALINE PHOSPHATASES, DESOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID CONTENTS OF TISSUES OF NORMAL AND SCORBUTIC GUINEA PIGS

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INTRODUCTION

A disturbance in carbohydrate metabolism has been observed in scorbutic guinea pigs (Banerjee and Ghosh, 1947). Phosphatases are enzymes intimately connected with carbohydrate metabolism. Reports concerning the relation of vitamin C to phosphatase activity appear to be very conflicting. Smith (1953) reported that during chronic scurvy serum phosphatase dropped below normal parallel to retarded growth. But Parkins and Zilva (1950) showed that the serum phosphatase of guinea pigs on a scorbutic diet began to fall after 12 days when growth was uninterrupted. They suggested that this fall was due mainly to scorbutic lesion and not to loss of weight. Harrer and King (1941) observed a moderate but irregular decrease in the phosphatase activity of intestinal mucosa and kidney cortex during severe scurvy, the decrease in brain and liver were not considered significant by them. Russell, Rouse and Read (1944) observed histochemically that scurvy did not bring about changes in the phosphatase activity of spleen, lungs and adrenals but a fall in kidney was observed. Gould and Shwachman (1942) also could not get any difference in the alkaline phosphatase activity of intestinal mucosa, kidney, adrenals or liver of scorbutic guinea pigs. Parkins and Zilva (1950) have shown that the phosphatase activity of liver, intestine or kidney of guinea pigs kept on a scorbutic diet for 15 days did not show any change, the kidney showed a fall in the premortal phase after 21 days.

In scurvy an impairment of wound healing has been observed by several workers (Wolbach, 1933, Bourne 1942, Bicknell and Prescott 1947). Toro (1952) observed that metabolism of nucleoprotein has an important role in healing of wounds. It is reasonable to suppose, therefore, that the effect of vitamin C is extended by way of the nucleoprotein metabolism. Gol'dshtein and Volkenzon (1941) have observed that in animal tissues the part of protein which is attached to iron-ascorbic acid complex, is nucleic acid. Gol'dshtein and his co-workers (1950, 1952) are of opinion that one of the main function of vitamin C is to form D.N.A. in cell nucleus. In avitaminosis C they have observed a decrease in D.N.A. and increase in R.N.A. in liver, adrenals and pancreas. They (1954) further corroborated their findings by the observation that the inclusion of P^{32} into D.N.A. of liver were reduced in scurvy. Rudas (1955) observed a decrease in both D.N.A. and R.N.A. of the granulation tissue in scurvy. But Fukuda and Shibatani (1953) have observed that in scorbutic liver, although there was a fall in protein nitrogen, the D.N.A. and R.N.A. remained constant.

Although alkaline phosphatase content of some tissues has been estimated in scurvy, we do not know of any work being carried out on the estimation of acid phosphatase under the same condition. Moreover all the work referred to above with the exception of Russell, Rouse and Read (1944) have

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been carried out biochemically. Histochemical methods have advantage over the biochemical methods in that the nuclear and cytoplasmic reactions can be differentiated as also the reaction in different zones. For all these reasons histochemical method of estimation for acid and alkaline phosphatases as also D.N.A. and R.N.A. were carried out in scorbutic and paired fed normal guinea pigs.

Material and Methods

The selection of the guinea pigs, feeding of the scorbutic diet either with or without supplement of ascorbic acid by the paired feeding technique were the same as those described previously (Banerjee and Deb, 1951). On the 21st day of the experiment, the animals were killed by decapitation. The tissues were taken out and pressed in a fold of filter paper to remove adherent blood and fixed overnight in chilled acetone and chilled 80 per cent alcohol. They were then dehydrated and embedded in paraffin. Alkaline phosphatase content of the acetone fixed tissues were measured by the histochemical method of Gomori (1946). Acetone fixed tissues were also used for estimation of acid phosphatase by the improved histochemical method of Gomori (1950). D.N.A. and R.N.A. in alcohol fixed tissues were measured histochemically by the method of Kurnick (1952). Feulgen reaction (Coleman 1938, Stowell 1946) for D.N.A. was also carried out in alcohol fixed tissues. Experiments were carried out with eight pairs of guinea pigs of both sexes. Histochemical reactions observed in the different tissues were similar in the animals of each group.

Observations

Acid and Alkaline Phosphatases

Pituitary : The cells of the pituitary showed presence of alkaline phosphatase activity; a reduction in activity in some portions of it has been observed in scurvy.

Acid phosphatase activity was present in the pituitary cells but the pattern of distribution did not change in scurvy.

Adrenals : In normal guinea pig, zona glomerulosa of adrenal cortex contained a high amount of alkaline phosphatase, the rest of the gland being negative. In scurvy accumulation of the enzyme has been observed in the zona fasciculata, there being no alteration in the rest of the gland.

Marked acid phosphatase activity has been observed in the zona glomerulosa and upper fasciculata of normal adrenals and a moderate activity in the lower fasciculata and zona reticularis. In scurvy, acid phosphatase completely disappeared from the fascicular zone, the glomerular zone showed no detectable change.

Testes : The nucleus of the cells of seminiferous tubules gave a positive reaction for alkaline phosphatase. In scurvy a decrease in enzymatic reaction has been observed.

The seminiferous tubular cells also contained acid phosphatase, there being no alteration of the reaction in scurvy.

Ovary : Pronounced activity was present in the germinal epithelium and also in the nucleus of the granulosa cells and the theca. In scurvy an overall reduction in enzymatic activity has been observed.

We could not detect any acid phosphatase activity either in normal or in scorbutic ovary.

Kideny : Alkaline phosphatase was present in the brush border of the proximal convoluted tubule in normal guinea pig. In scurvy a reduction in the enzymatic activity has been observed.

Acid phosphatase activity was widely distributed in various portions of kidney tubules and glomerulus. In scurvy an overall augmentation in activity has been observed.

Liver : Like Gomori (1941) we could not observe any alkaline phosphatase activity in normal guinea pig liver; in scurvy also we have observed absence of enzymatic activity.

Hepatic cells contained considerable amount of acid phosphatase. In scurvy possibly there was a slight decrease in activity.

Pancreas : Both the acinar and islet cells were devoid of alkaline phosphatase activity and scurvy did not bring about any alteration in it.

Acinar and islet cells contained a moderate amount of acid phosphatase. no alteration in staining intensity has been observed in scurvy.

Desoxyribonucleic Acid (D.N.A.) and Ribonucleic Acid (R.N.A.)

A pronounced reduction in D.N.A. has been observed in scorbutic testes and ovary. Adrenals, kidney, pancreas and possibly liver also showed a decrease. The pituitary did not show any change, or there might have been an increase in reaction.

R.N.A. showed an increase in kidney, zona reticularis of adrenals, liver and possibly pancreas. Pituitary, testes and ovary did not show any change in staining intensity.

Discussion

Acid and Alkaline Phosphatases

Pituitary : The alkaline phosphatase activity of the pituitary is likely to be hormonally controlled. Karkun and Kar (1954) have observed a pronounced increase in alkaline phosphatase activity of the basophil cells of pars intermedia of cat's pituitary on injection of A.C.T.H. We have observed a reduction in alkaline phosphatase activity in some portions of anterior pituitary in scurvy but can not say at present if this is due to reduction in some particular cell type. It may be possible that the reduction is due to alteration in some particular pituitary 'trophic' hormone in vitamin C deficiency.

Adrenals : It is seen that adrenal acid and alkaline phosphatases behave differently, there being an increase in alkaline and decrease in acid phosphatase in the zona fasciculata of scorbutic adrenals. Moog (1946) and also Dempsey and Wislocki (1946) remarked that adrenal alkaline phosphatase plays an important rôle in the metabolism of lipoids; and so possibly in the synthesis of adrenal cortical hormone. Kar (1953) has observed disappearance of alkaline phosphatase from the zona fasciculata of cat's adrenals after A.C.T.H. treatment. We have observed a decrease in cholesterol and ascorbic acid of adrenals (Banerjee and Deb, 1951) which together with other findings (Banerjee and Deb, 1952, 1952*a*) led us to conclude a state of hypofunction of adrenal cortex in scurvy. The accumulation of alkaline phosphatase in the zona fasciculata in scurvy may be due to its non-utilization. This finding is contradictory to that of Russell, Rouse and Read (1944) and also that of Gould and Shwachman (1942).

The rôle of acid phosphatase in adrenals is not clearly known and so the observed disappearance of it in scurvy can not be explained.

Testes : The testicular alkaline phosphatase is in someway related to testicular function. Kar (1950, 1951) has shown that injection of desoxycorticosterone acetate either to sparrows or pigeons causes a decrease in testicular alkaline phosphatase together with a suppression of testicular activity. It may be possible that the decrease in alkaline phosphatase of scorbutic testes is due to some altered physiological function in it.

Ovary : Kar (1953) has reported a decreased alkaline phosphatase activity in germinal epithelium and luteal cells of rat's ovary after adrenalectomy and ascribed it to be due to reduced activity. The overall reduction observed in scorbutic ovary might have a similar cause. The hypofunction of adrenal cortex observed in scorbutic guinea pigs (Banerjee and Deb, 1952*a*) might have a similar effect on the alkaline phosphatase activity of ovary as that of adrenalectomy on rat ovary.

Kidney : Like adrenals, kidney acid and alkaline phosphatases behave differently. In scurvy a fall in alkaline and increase in acid phosphatase have been observed. Kochakian (1947) similarly observed in rodent kidney an increase in acid and decrease in alkaline phosphatase on testosterone administration. Kochakian and Dontigny (1948) have observed in non-functional kidney, a decrease in alkaline phosphatase activity and Kay (1926) believed that the renal alkaline phosphatase is related to renal function. The dye trypan blue has an affinity for diseased cells and nacrotic tissue. Russell and Callaway (1943) have reported increased deposition of trypan blue in the proximal convoluted tubule in scurvy. The decrease in alkaline phosphatase observed in scorbutic kidney might therefore be due to a decrease in functional activity. Whereas our observation confirms the histochemical findings of Russell, Rouse and Read (1944) and the biochemical findings of Harrer and King (1941) also of Parkins and Zilva (1950), it is contradictory to that of Gould and Shwachman (1952) who have observed no alteration in kidney phosphatase activity.

Davison Reynolds, Barrueto and Lemon (1954) have shown that prostatic acid phosphatase is responsible for transphosphorylase activity, transfering phosphate to suitable alcohols. It may be possible that the raised acid phosphatase in scurvy indicates a rise in phosphorylating capacity in kidney.

Liver : The guinea pig liver is practically devoid of alkaline phosphatase which has also previously been observed by Gomori (1941).

Noerberg (1950) suggested a relationship between liver acid phosphatase and protein synthesis. Wachstein (1945) observed a decreased acid phosphatase activity in the liver of protein depleted animals. The reduced hepatic acid phosphatase observed in scurvy might have a relationship with the decreased liver protein reported by Fukuda and Shibatani (1953).

Desoxyribonucleic Acid (D.N.A.) and Ribonucleic Acid (R.N.A.)

In vitamin C deficiency an opposite trend as regards D.N.A. and R.N.A. contents of tissues have been observed. The D.N.A. content of all the tissues with the exception of pituitary, decreased, whereas the R.N.A. content of kidney, liver, adrenals and possibly pancreas increased. No change has been observed in the R.N.A. contents of testes and ovary as also D.N.A. and R.N.A. contents of pituitary.

Gol'dshtein and his co-workers (1950) are of opinion that vitamin C regulates the viscocity and formation of D.N.A. in the cell nucleus. In avitaminosis C, due to deficiency of vitamin C in the tissues examined, there was less formation of D.N.A. as revealed by the histochemical method, observed by us. Our results confirm the biochemical observation of Gol'dshtein and his co-workers (1950, 1952) who have also observed a decrease in liver, adrenals, and pancreas. It may be possible that in scurvy there was no alteration in the ascorbic acid content of pituitary which can account for the stability of D.N.A. in it.

Caspersson (1950) is of opinion that nucleic acids take part in the synthesis of cellular proteins not only in the nucleus but also in the cytoplasm. Danielli (1953) remarked that the exact relationship of nucleic acids to protein synthesis is unknown. Nucleic acids do not participate in the synthesis of polypeptide chain, the first step in protein production. After polypeptides are formed nucleic acids

may act either as a trapping and/or folding agent in protein formation. So in the absence of adequate supply of polypeptides, R.N.A. can not help in protein synthesis. In vitamin C deficiency a fall in total nitrogen content of blood has been observed (Banerjee 1957). It is possible therefore that in this condition the tissues concerned in protein synthesis are not adequately supplied with polypeptides. The R.N.A. present in the particular tissue is not utilized to synthesise protein and so it is present in higher concentration in some tissues. Unlike D.N.A., vitamin C has no action in the formation of R.N.A., as in vitamin C deficiency it is seen to be present in normal, even in higher amount. Our observation of the increased R.N.A. content of certain tissues also confirms the biochemical observation of Gol'dshtein and his co-workers (1950, 1952).

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Summary

1. Acid and alkaline phosphatases as also D.N.A. and R.N.A. contents of Pituitary, Adrenals, Testes, Ovary, Kidney, Liver and Pancreas have been estimated histochemically in scorbutic and paired fed normal guinea pigs.

2. In scurvy a decrease in alkaline phosphatase has been observed in Testes, Ovary, Kidney and some portions of Pituitary, whereas an increase has been observed in the Fascicular zone of Adrenals. Liver and Pancreas did not show any change.

3. Acid phosphatase showed an increase in Kidney and a decrease in Fascicular zone of Adrenals and possibly Liver in scurvy. No alteration in enzymatic activity has been observed in other tissues examined.

4. Reduction in D.N.A. has been observed in scorbutic Testes, Ovary, Adrenals, Kidney, Pancreas and possibly Liver. Pituitary did not show any change or there might have been an increase in it.

5. In scurvy R.N.A. showed an increase in Kidney, zona Reticularis of Adrenals, Liver and possibly Pancreas. No detectable change has been observed in Pituitary, Testes and Ovary.

6. The changes observed have been ascribed to be due to alterations in hormonal and metabolic factors in scurvy.

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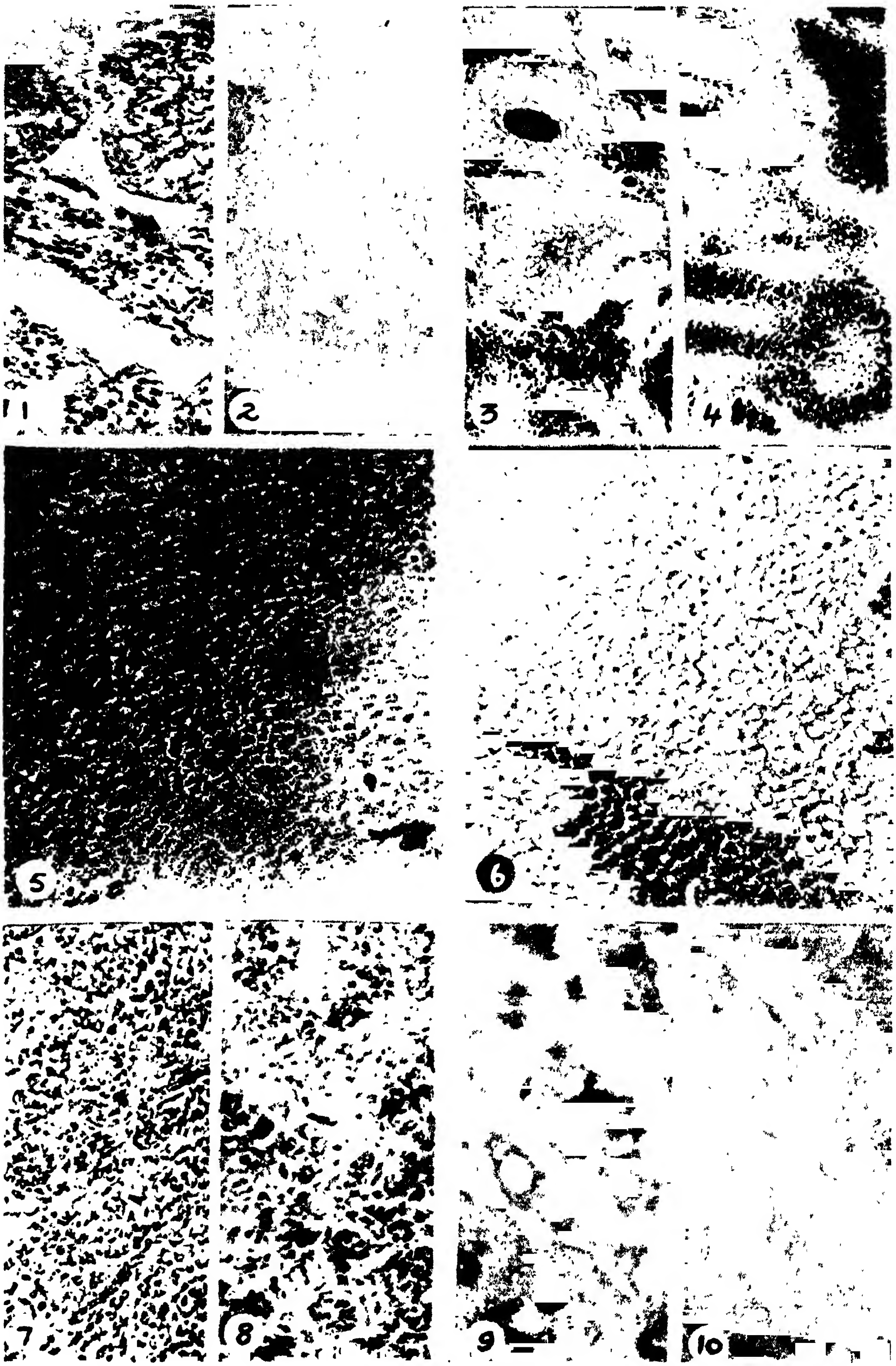
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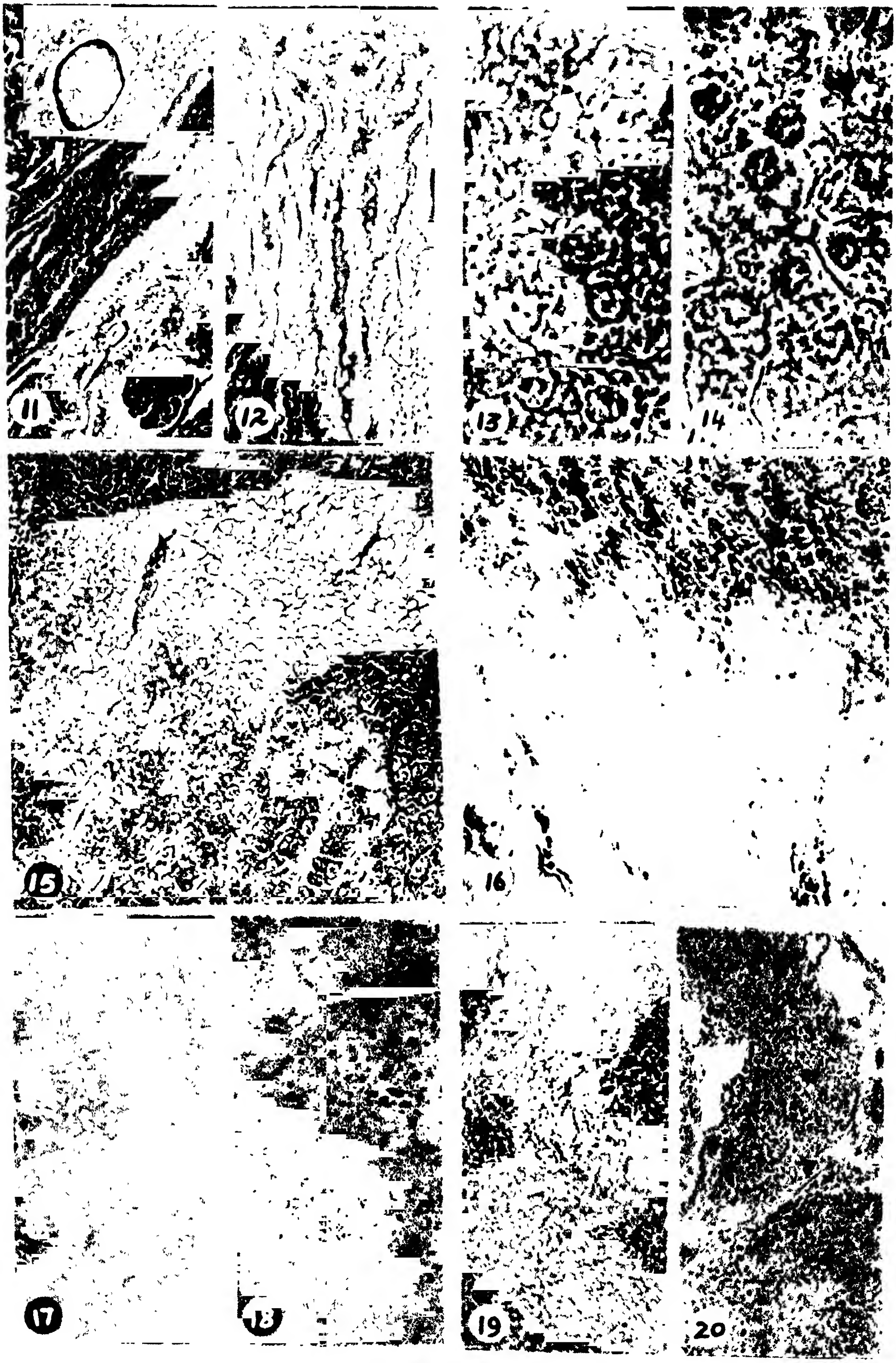
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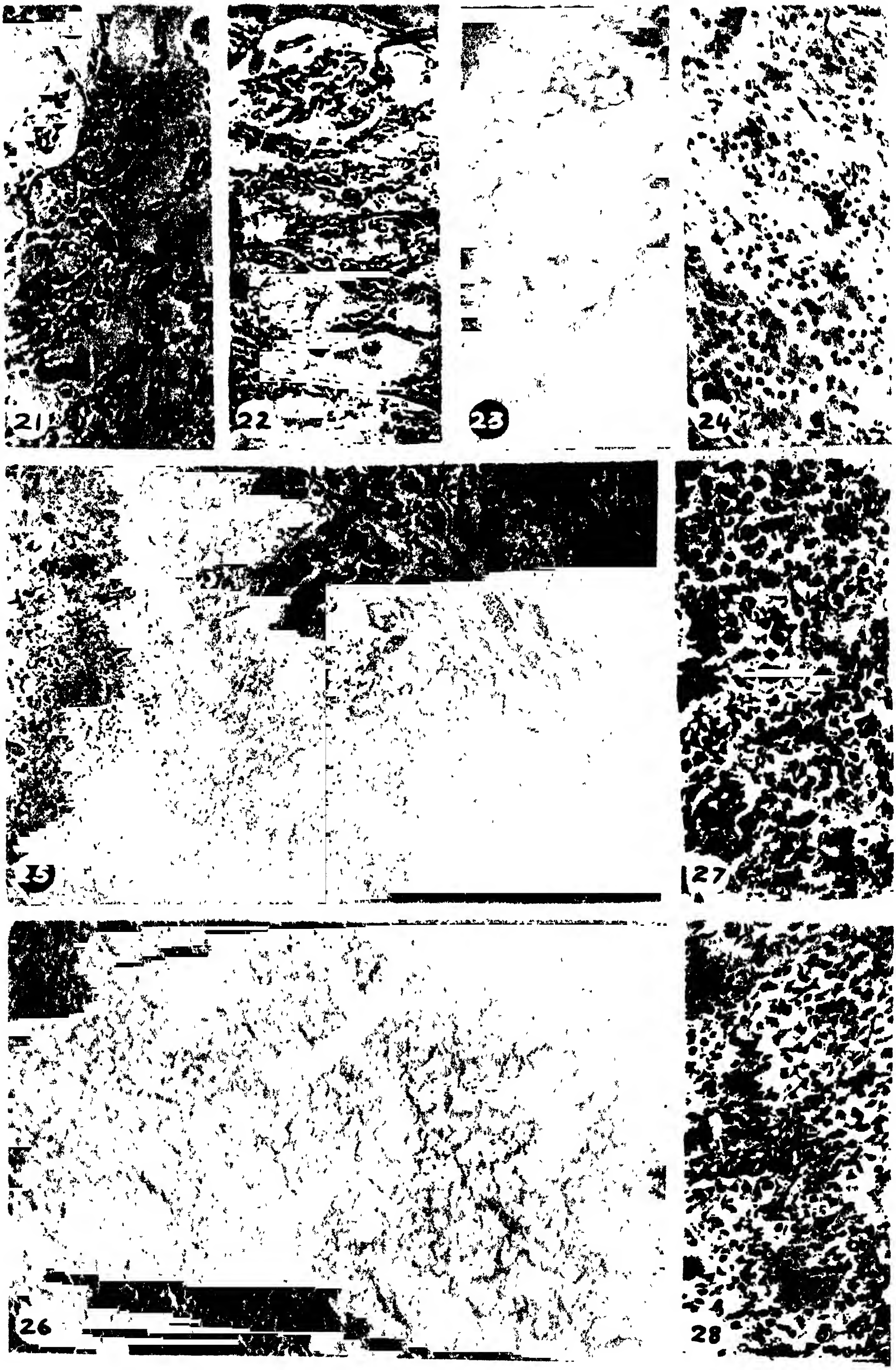
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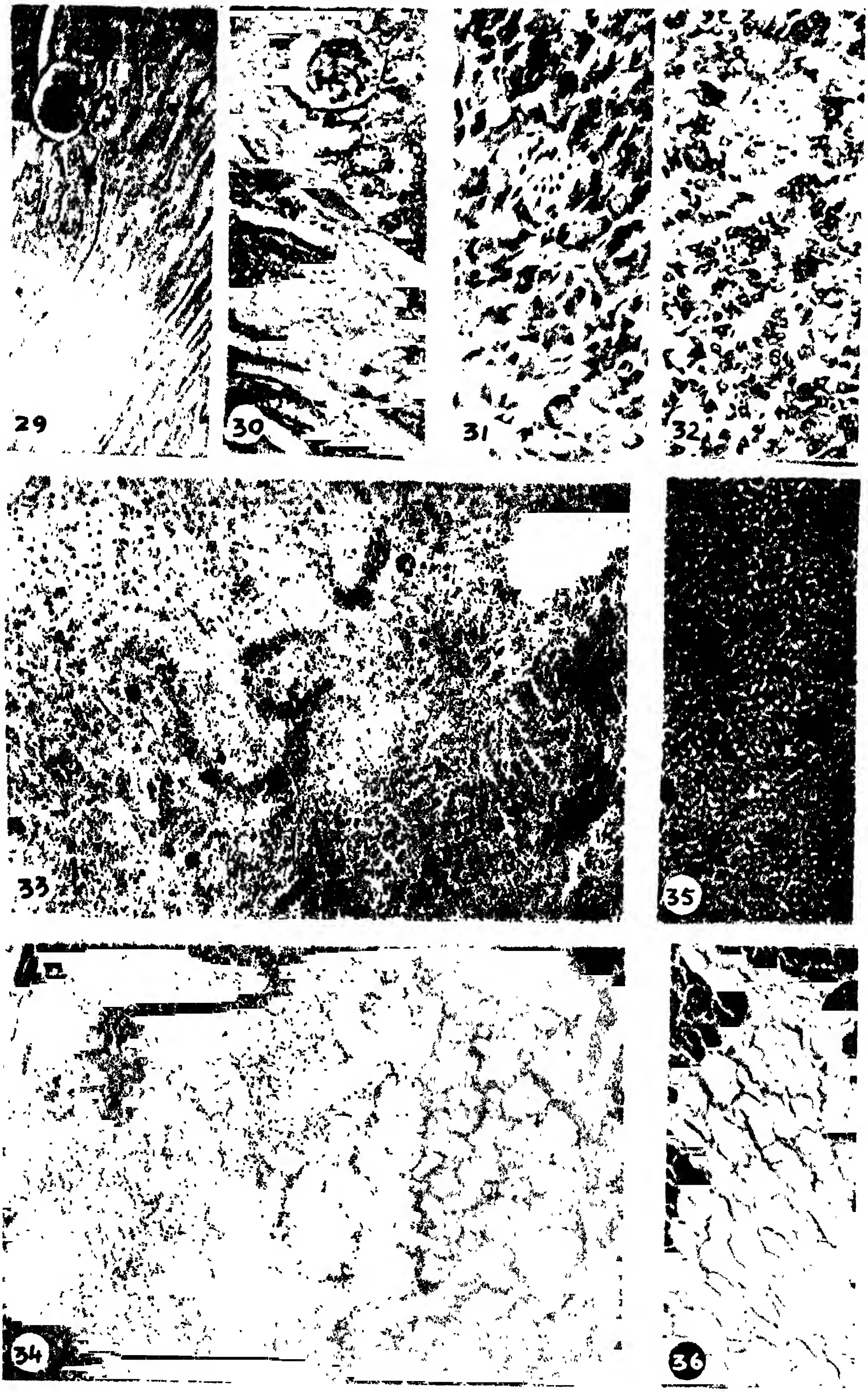
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Description of Plates

Plate I

Fig. 1. Normal testes, alkaline phosphatase. Pronounced reaction in the nucleus of seminiferous tubular cells. × 75.
Fig. 2. Scorbutic testes, alkaline phosphatase. A reduction in enzymatic reaction. × 75.
Fig. 3. Normal ovary, alkaline phosphatase. Enzymatic activity in ovarian cells. × 60
Fig. 4. Scorbutic ovary, alkaline, phosphatase. A decrease in staining intensity. × 60.
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Plate II

Fig. 11. Normal kidney, acid phosphatase. Enzymatic activity in glomerulus and tubules. × 60.
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Fig. 13. Normal liver, acid phosphatase. Moderate reaction in hepatic cells. × 150
Fig. 14. Scorbutic liver, acid phosphatase. Reduction in enzymatic activity. × 150
Fig. 15. Normal adrenal, acid phosphatase. Predominant activity in zona glomerulosa and upper fasciculata. Moderate activity in lower fasciculata. × 60.
Fig. 16. Scorbutic adrenal, acid phosphatase. Absence of enzymatic activity in zona fasciculata. × 60.
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Fig. 18. Scorbutic testes, D.N.A. Feulgen. × 75.
Fig. 19. Normal ovary, D.N.A. Feulgen. × 75.
Fig. 20. Scorbutic ovary, D.N.A. Feulgen. × 75.

Plate III

Fig. 21. Normal kidney, D.N.A. Feulgen and Light Green. × 150.
Fig. 22. Scorbutic kidney, D.N.A. Feulgen and Light Green. × 150.
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Fig. 27. Normal pituitary, D.N.A. Feulgen. × 150.
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Plate IV

Fig. 29. Normal kidney, R.N.A. Methyl Green—Pyronin. × 75.
Fig. 30. Scorbutic kidney, R.N.A. Methyl Green—Pyronin Increase in staining intensity. × 75.
Fig. 31. Normal pancreas, R.N.A. Methyl Green—Pyronin. Pronounced staining of the acinar cells. × 150.
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Fig. 33. Normal adrenal, R.N.A. Methyl Green--Pyronin. × 75.
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Fig. 36. Scorbutic liver, R.N.A. Methyl Green Pyronin. Increased staining of the hepatic cells. × 150.

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OXYGEN REQUIREMENTS OF FRY OF THE INDIAN MAJOR CARP, *LABEO ROHITA* (HAMILTON)

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(Communicated by B. S. Bhimachar, F.N.I.)

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Contents

Introduction

In view of a general awakening to the culture of fish in various parts of the country, Central Inland Fisheries Research Station organised a Fish Seed Syndicate at Calcutta in 1952 for supply of fry and fingerlings of Indian major carps to deficit States for piscicultural purposes. But, for successful execution of any large scale programme of fish culture, it is essential to evolve improved methods of transport of fry over long distances. It was therefore considered necessary to obtain data on oxygen requirements of fry which would be of basic importance in devising such methods. The present paper communicates the results of experiments relating to oxygen requirements of fry of *Labeo rohita* (Hamilton) under laboratory conditions.

Material and Methods

Fry of *L. rohita*, ranging in total length between 22 and 46 mm, were supplied by the Syndicate from their nurseries which were being maintained along scientific lines. On arrival in the laboratory the fry were immediately placed, 500 to a container, in large, hardened earthen containers of 15-gallon capacity and acclimatised to the chlorine-free corporation tap water of *p*H 8.0 - 8.2 The dissolved oxygen of water ranged between 6.0 and 6.4 p.p.m. and temperature between 27.0 and 30°.0C. Free chlorine from the water was eliminated by vigoroulsy agitating it over a period of several hours. Tap water subjected to this treatment and kept overnight was used in all the experiments described here. Planktonic organisms (Diatoms, Desmids, Rotifers, Copepods etc.) in proper quantities were given to the fry once in 48 hours. The feeding times were so regulated, that at least 24 hours lapsed between them and the time when the fry were used in experiments. Only normal healthy specimens were selected from the stock and no fish kept in the containers for more than six days were used in these experiments.

Oxygen was determined by the basic unmodified Winkler's method as recommended by American Public Health Association (1946).

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EXPERIMENTS

(i) *One-litre sealed bottles*

Two series, each having twelve 1-litre reagent glass bottles, were filled with water of known oxygen content. A fixed number of fry, all of about the same size, was introduced in each bottle and the bottle sealed immediately. The bottles in one series only were covered with thick opaque paper to shut off penetration of light. Two bottles, one each from the two series, were unsealed at 20-minute intervals and samples of water collected for determination of dissolved oxygen. The test fry from each bottle were removed immediately, fixed in 5 per cent formaldehyde, dried on a blotting paper and weighed to the nearest milligram. The experiments in these series were repeated four times. The results of the four series of experiments are summarised in Table I.

TABLE I

Summarised results of the four series of experiments in closed bottles

Serial number of bottle	Duration of experiment in minutes	Oxygen consumed (in mg.m) by 100 gm. of *L. rohita* fry per hour — A Covered series: Exp. I-A	Exp. II-A	Exp. III-A	Exp. IV-A	B Exposed series: Exp. I-B	Exp. II-B	Exp. III-B	Exp. IV-B
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	20	69.06	64.23	68.17	69.61	65.76	65.90	68.69	70.52
2	40	66.42	69.79	69.01	69.32	68.16	68.17	66.69	71.12
3	60	70.26	66.36	67.03	69.09	64.29	68.92	70.61	66.92
4	80	64.68	61.82	63.42	66.91	69.36	69.64	60.78	56.10
5	100	68.16	65.56	65.16	67.51	62.82	58.04	65.57	65.11
6	120	60.39	59.85	67.45	65.40	67.98	64.86	64.11	62.71
7	140	55.38	60.36	59.26	60.60	64.02	56.83	62.91	67.55
8	160	52.95	54.77	59.13	54.31	61.56	59.29	58.29	60.09
9	180	55.53	53.16	52.92	51.60	64.77	64.74	61.26	64.51
10	200	51.37	51.62	54.26	50.40	68.34	62.54	56.84	66.91
11	220	50.43	48.75	51.11	52.51	54.81	59.19	61.89	-
12	240	51.06	52.26	51.47	52.20	59.28	63.48	58.68	
Temperature °C	C	29.60	28.20	28.40	28.80	29.60	28.10	28.20	28.80
	T	30.50	29.00	30.20	29.40	30.60	29.30	29.80	29.80
D.O. of the medium (p.p.m.)	C	6.70	6.75	6.58	6.72	6.68	6.75	6.58	6.72
	T	2.32	2.41	2.09	1.98	1.93	1.89	1.82	1.98
Size range of fry (mm.)		17–30	17–30	17–30	16–31	17–30	17–30	17–30	17–30
Weight range in fry in each bottle (gm.)		1.89–3.12	1.97–2.72	1.78–3.15	1.69–3.25	1.81–2.35	1.78–3.05	1.73–2.98	1.60–2.79

C = At the commencement of experiments.
T = At the termination of experiments.

(*ii*) *Continuous-flow system*

The apparatus used in this series was similar in design and operation to that of Keys (1930) except that in the present apparatus five respiratory chambers instead of two were provided and were all properly covered with thick opaque paper. The flow of water in the chambers was maintained at a rate, which was considered slow enough to enable measurement of the amount of oxygen removed by test fry and also sufficient to maintain a supply of fresh water in the chambers, so that diminished oxygen would not enter as a limiting factor. The fry remained in the chambers for full two hours before observations were taken. Once the observations were commenced, water from the respiratory and control chambers was allowed to pass into collecting jars for exactly 20 minutes and then by-passed to the sink. Known volume of water from all the collecting jars was taken for determination of dissolved oxygen and the balance drained off and measured. The by-pass lines were then shut off and the whole procedure begun again, this being repeated from 8 to 10 times over a period of about 10 hours. At the conclusion of the last observation, fry were removed from each chamber, fixed in 5 per cent formaldehyde and weighed. A preliminary check on pH and CO_2 of water, entering and leaving the respiratory chambers, showed no significant change. Temperature of water during the experiments ranged between 28°.2 and 30°.4C.

Results of twenty-one sets of experiments are summarised in Table II. In each set, at least 8-10 observations each of 20 minutes' duration were made over a period of at least 10 hours. It can be seen clearly that the values of respiration rate, obtained in 8-10 observations, do not show any significant difference and therefore the mean of all the values in a particular experiment has been calculated to show the average respiration rate for 20-minute period.

Discussion

The method of measuring respiration rates in sealed bottles has been criticized specially when the duration of experiments is rather long, because of the possible accumulation of CO_2 and other katabolic products in the experimental bottles. The other serious defect in this type of experiment is the progressive depletion of oxygen in the water and consequent reduction in the oxygen available for respiration. At the end of four hours (maximum period allowed in bottle experiments) the dissolved oxygen of the medium was never less than 1.82 p.p.m. (cf. Table I) and since Basu (1949) has stated that Indian carp fry can normally live for at least 24 hours without any apparent ill-effects in water with D.O. between 1 and 1.5 p.p.m., it would seem unlikely that the depleted oxygen in the experimental bottles would have been a limiting factor for normal respiration.

The first point of interest that emerged from "sealed bottle" experiments was that the values for the first hour or so, showed a persistent increase over subsequent values. Keys (1931) experimenting with *Fundulus parvipinnis*, observed that the maximum respiratory exchange was attained in the first two or three hours and the rate subsequently tended to be constant. According to Keys (*op. cit.*) at least part, if not all, of the explanation of the phenomenon must be that in the excitement and struggling incident to transfer to the respiratory chambers, the fishes acquire a considerable "oxygen debt". Wells (1932) and Black *et al.* (1939) among others, have also recorded that handling of fish raises their oxygen consumption well above the resting level and that the excited condition may last for some time even after the fish is not visibly active. Graham (1949) calculating the maximum active and standard respiratory rates of speckled trout (*Salvelinus fontinalis*), noted that the handling of the fish, as it was first put into a chamber, was evidently sufficient stimulus to activity and need for further agitation during the next hour

TABLE II

Results of experiments in continuous-flow system apparatus showing oxygen consumption rates of fry of Labeo rohita *(Ham.) at resting level*

Sl. No.	No. of fish per chamber	Size range mm.	Average size mm.	Combined wt. of fish per chamber (gm.)	Number of 20-minute observations	Oxygen consumed in 20-minutes (mgm.)		Mean oxygen consumed in 20-minute period (mgm.)
(1)	(2)	(3)	(4)	(5)	(6)	(7)		(8)
1.	15	23—27	25.50	1.900	8	0.319 0.321 0.320 0.340	0.318 0.325 0.332 0.327	0.325
2.	15	23—27	26.13	1.941	8	0.391 0.366 0.357 0.350	0.390 0.381 0.372 0.360	0.371
3.	5	37—42	38.80	2.412	9	0.397 0.358 0.371 0.368 0.373	0.382 0.368 0.367 0.375	0.373
4.	5	37—42	40.00	2.690	10	0.533 0.491 0.504 0.489 0.507	0.490 0.498 0.504 0.508 0.498	0.502
5.	26	23—28	25.90	2.941	10	0.444 0.424 0.469 0.508 0.490	0.502 0.478 0.490 0.467 0.462	0.473
6.	20	25—28	26.25	3.095	10	0.486 0.515 0.545 0.555 0.540	0.490 0.498 0.530 0.528 0.532	0.522
7.	5	39—46	44.00	3.100	8	0.552 0.535 0.535 0.511	0.522 0.550 0.546 0.538	0.536
8.	14	27—33	29.70	3.220	9	0.616 0.616 0.641 0.570 0.614	0.620 0.602 0.614 0.610	0.611
9.	30	23—28	25.90	4.272	8	0.796 0.726 0.758 0.790	0.789 0.776 0.746 0.796	0.772
10.	8	37—42	39.10	4.322	10	0.590 0.625 0.636 0.638 0.652	0.642 0.614 0.652 0.644 0.614	0.632

TABLE II—(*contd.*)

Sl. No.	No. of fish per chamber	Size range mm.	Average size mm.	Combined wt. of fish per chamber (gm.)	Number of 20-minute observa-tions	Oxygen consumed in 20-minutes (mgm.)	Mean oxygen consumed in 20-minut period (mgm.)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
11.	10	35—42	38.10	4.743	10	0.825 0.846 0.808 0.836 0.860 0.842 0.795 0.820 0.830 0.806	0.827
12.	25	26—33	30.16	5.652	9	0.944 0.948 0.930 0.918 0.891 0.942 0.967 0.944 0.960	0.938
13.	40	23—28	26.10	5.940	10	0.996 0.927 0.985 0.936 0.924 0.964 0.930 0.938 0.912 0.946	0.946
14.	10	38—44	41.10	6.103	8	0.987 0.961 0.961 0.973 0.958 0.955 0.948 0.962	0.963
15.	10	23—34	26.30	6.661	10	1.012 1.146 1.138 1.118 1.065 1.158 1.214 1.162 1.100 1.146	1.126
16.	15	36—42	38.60	6.930	10	1.128 1.248 1.245 1.234 1.172 1.241 1.203 1.250 1.200 1.225	1.215
17.	60	22—29	25.43	8.090	9	1.462 1.430 1.494 1.448 1.516 1.536 1.530 1.503 1.518	1.493
18.	20	34—40	36.60	8.402	9	1.522 1.575 1.590 1.558 1.590 1.582 1.540 1.536 1.532	1.558
19.	60	24—30	26.05	8.500	10	1.466 1.451 1.439 1.492 1.470 1.478 1.456 1.495 1.500 1.465	1.471
20.	20	36—45	41.10	11.530	10	1.915 1.935 1.887 1.905 1.935 1.896 1.787 1.890 1.933 1.924	1.910
21.	20	37—46	40.70	11.942	10	1.887 1.998 1.827 1.872 1.899 1.868 1.816 1.896 1.814 1.906	1.878

or so, which was otherwise necessary to obtain the maximum active respiratory rate, was not felt.

It is thus obvious from the results obtained in "sealed bottle" experiments that the values of at least the first three to five observations represent maximum active respiratory rate. The subsequent values are lower and although on the whole steady, these cannot possibly be representative of respiratory rate at resting stage of fish because these values are computed averages of all the observations made in a particular series and therefore include the values representative of maximum active metabolism also. By prolonging the duration of experiments in sealed bottles, it would be perhaps possible to obtain values of respiratory rate at resting stage, but in that case the possibility of progressive depletion of oxygen as a limiting factor for normal respiration, will have to be taken into consideration. Thus the "bottle" experiments, at best, can only give an idea of active respiratory rates.

The other point of interest was that the values on respiratory rates obtained in the "uncovered" series of "bottle" experiments, although rather inconsistent (Table I, panel B.), were nevertheless comparable to those obtained in the initial stages of experiments in "covered" series which represent active respiratory rate. As has already been stated, the active respiratory rate is attributable to the handling of fish and since subsequent values in the "uncovered" series were also comparable to initial values, it follows that in these series the fry were in a constant state of excitement even to the end of experiments. The state of excitement evinced by fry in "uncovered" series may perhaps be ascribed to very frequent and irregular variation in the intensity of light in the experimental bottles incident to constant and continuous movement of workers in the room.

In the experiments conducted in continuous-flow system, the test fry were therefore allowed to remain in the respiratory chambers for a period of two hours before the observations were taken. It may also be noted that all the respiratory chambers were properly covered with thick opaque paper so that external movement could not act as a stimulus. It is therefore reasonable to assume that the values obtained in these experiments represent respiratory rate at resting stage of fry. Since the difference in the respiratory rates (on the basis of 20-minute observations) among 8-10 observations made in a particular series, was found to be insignificant (cf. Table II) it would appear that the oxygen consumption of *L. rohita* is, perhaps a constant function under controlled laboratory conditions, at least within the duration of present experiments. Glausen (1936) experimenting with fresh water fishes, however, concluded that the rate of oxygen consumption varies from hour to hour in the same fish and shows differences between individuals of the same species.

The respiratory chambers provided in the continuous-flow system were of the same volume (one litre) and the number of individuals in each chamber varied from 5 to 60 (cf. Table II) so that the volume of water available to an individual was not always the same. It is therefore natural to expect that grouping of fish would, perhaps, depress the respiratory rate (Schuett, 1933; Schlaifer, 1939). But the results seem to show that the respiratory rate is dependent only on the mass of fishes irrespective of the number of fry in a chamber. Consequently the volume of space available to an individual fish in a chamber cannot be considered a limiting factor for normal respiration, at least within the size range and the number of individuals used in these experiments.

The results of twenty one sets of experiments summarised in Table II, show that the oxygen consumption rate of fry of *L. rohita* increases with increasing weight. It has therefore been possible to reduce the data to a mathematical formula as follows :

$$\log O_2 = 2.24764 + 0.9667676 \log W/10^3 \qquad \ldots \quad (i)$$

where O_2 = oxygen consumed (mgm.) in 20 minutes at resting stage of respiration, and

W = combined weight of fry (gm.) in a respiratory chamber.

To test whether the difference between observed and theoretical values, as calculated from formula (i), is significant or not, χ^2 ("chi square") test was made. The value of χ^2 being 0.516 with 20 d.f. (Table III) it follows that the fitted curve represents the observed data. The small value of χ^2 may presumably be attributable to the counter balance of the positive and negative errors with each other.

TABLE III

Comparison of observed and calculated values of oxygen consumed by L. rohita *(Ham.) fry in 20 minutes*

($\log O_2 = 2.24764 + 0.9667676 \log W/10^3$)

Exp. No.	Weight of test fish (gm.)	Observed values of O_2 consumed in 20-minutes (mgm.)	Calculated values of O_2 consumed in 20 minutes (mgm.)	P.C. difference*	χ^2 test
(1)	(2)	(3)	(4)	(5)	(6)
1.	1.900	0.325	0.329	1.230	
2.	1.941	0.371	0.336	9.434	
3.	2.442	0.373	0.419	12.332	
4.	2.690	0.502	0.460	8.367	
5.	2.911	0.473	0.497	5.074	
6.	3.095	0.522	0.527	0.958	
7.	3.100	0.536	0.528	1.487	
8.	3.221	0.611	0.548	10.311	
9.	4.272	0.772	0.720	6.736	
10.	4.322	0.632	0.728	15.190	
					χ^2 = 0.516
11.	4.743	0.827	0.797	- 3.628	
12.	5.652	0.938	0.944	0.640	
13.	5.940	0.946	0.990	4.640	
14.	6.103	0.963	1.016	- 5.504	
15.	6.661	1.126	1.106	1.776	
16.	6.900	1.215	1.149	- 5.433	
17.	8.090	1.493	1.335	-10.582	
18.	8.402	1.558	1.385	-11.104	
19.	8.500	1.471	1.400	- 4.827	
20.	11.530	1.910	1.880	1.571	
21.	11.942	1.878	1.945	3.567	

*Difference between calculated and observed values expressed as percentage of the latter.

For practical utility of the results, it was considered necessary to study length-weight relationship of fry of *L. rohita*, since the trade actually engaged in transport of fry in oxygen containers, understands better, if the number and size of fry to be transported in a container are given, rather than their combined weight. For this purpose over 300 fry of *L. rohita*, ranging in size between 21 and 45 mm. in total length, were examined. The fry for this study came from the nursery tanks

which provided material for the experiments described above. The correlation between length and weight is found to be of high magnitude with the value of r being 0.96368. The general relationship has been calculated from the formula $W = CL^n$, C and n being determined by the method of least squares. The relationship is expressed by :

$$W = 0.205385 \times 10^{-5} \times L^{3.432022} \qquad \ldots \quad \text{(ii)}$$

In the case of young and adult of *L. rohita* from ponds, Jhingran (1952) found the relationship to be

$$W = 1.554 \times 10^{-5} \times L^{3.0140028}$$

On the basis of the two equations (i) and (ii) above the oxygen requirements per hour of 1000 fry of different size ranges at the resting stage and at the active stage of respiration has been calculated and given in Table IV.

TABLE IV

Oxygen consumption per hour by 1,000 fry of L. rohita *(Ham.) of different size ranges at resting and active levels of respiration*

Length of fry (mm.)	Weight of 1000 fry (gm.)	Oxygen consumption per hour by 1000 fry at: Resting level, mgm.	Resting level, litre at 35°C and normal pressure	Active level, mgm.	Active level, litre at 35°C and normal pressure
(1)	(2)	(3)	(4)	(5)	(6)
20	60	27.31	0.0191	40.57	0.0284
25	123	55.99	0.0391	83.18	0.0582
30	240	109.25	0.0765	162.31	0.1136
35	398	181.17	0.1268	269.16	0.1884
40	645	293.60	0.2055	437.21	0.3060

For calculation of active respiratory rate, averages of all the values obtained in the first 60 minutes in "covered" series of bottle experiments, were taken into consideration.

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Summary

Oxygen consumption rates of fry of *Labeo rohita* (Ham.), ranging in sizes from 22 to 46 mm., at resting and active stages of respiration have been studied. It was observed that oxygen consumption rate appeared to be dependent on the total mass of test fry rather than their number, suggesting that "grouping" effect on the respiratory rate was not apparent. The data have been reduced to a mathematical equation : $\log O_2 = 2.24764 + 0.9667676 \log W/10^3$ (where O_2 is the oxygen consumed in mgm. at resting level in 20 minutes and W is the combined weight of fry in grams). For practical utility of these results, length-weight studies relating to fry of *L. rohita* were made by examining over 300 specimens. The relationship is expressed as : $W = 0.205385 \times 10^{-5} \times L^{3 \cdot 132022}$. On the basis of the two equations, a table has been prepared for guidance of the trade engaged in transport of fry, which depicts the oxygen requirements (in volume) at resting and active stages of respiration of 1000 fry of sizes between 20 mm. and 40 mm. at normal pressure and 35°C.

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EFFECT OF RAINFALL ON THE YIELD OF RICE AND EVALUATION OF WATER REQUIREMENT

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Introduction

Geographical distribution of rice-growing areas in the world over regions of heavy rainfall in the relatively short period of the cropping season indicates that water supply is probably the chief limiting factor to the growth and production of rice. Knowledge about water requirement of rice is, however, meagre and estimates are quite variable. In Japan, water requirement was estimated to vary from 27.6 to 51.5 acre inches (Leonard, 1948) and in Thailand, it was 72 acre inches (Grist, 1953). In Louisiana and Texas, 38 to 60 acre inches of water (Fortier. 1926) and in California, 60 to 72 acre inches were estimated to be required for the crop. According to Roe (1950), water requirement varied from 48 to 93 acre inches while Robertson's reports on irrigation experiments in Biggs indicated that average annual use of water for the 3-year period was 54 acre inches. In India with seventy-five million acres under this crop constituting about twenty-two per cent of her cultivated lands, there has hardly been any work on water requirement of rice except determination of the transpiration ratio (Hector. 1925–28; Singh *et al.*, 1935; Ganguli, 1950; Ghose *et al.*, 1956).

An attempt has been made to evaluate the water requirement of transplanted rice from the effect of rainfall on yield. Determination has been made of the water requirement of the crop for the entire growing season as well as the different stages of its growth and maturity, and of the precise level of soil moisture suitable for promotion of high yield. Loss of moisture from rice fields by way of transpiration, evaporation, percolation and run off have been worked out and correlated with water requirement. Physiological significance of the depth and frequency of rainfall in flowering and fertilisation processes and the nutritional rôle of nitrogen supplied by rain water along with probable mechanism of its absorption and utilisation have been discussed.

Effect of Rainfall on Yield of Rice

In course of manurial experiments on rain-fed transplanted rice, it was observed (Basak, 1956) that even under apparently identical conditions, there was considerable variation in the magnitude of yield obtained in different years from same sets of plots under same sets of treatments and it was related to rainfall feeding the crop from transplatantion to harvesting (Table 1 and Fig. 1). In fact, progressive increase in rainfall induced a fairly corresponding increase in yield of grain and the effect of rainfall on yield was statistically significant. These results gave a clue to the present investigations. It was argued that if data on grain yield of rice grown over a large number of years in regions under varying magnitude of rainfall could be obtained along with corresponding data on rainfall feeding the crop, it might possibly reveal a close correlation between yield and rainfall and thereby suggest a way for evaluation of the water requirement of rice.

TABLE 1

Average yield of rice (Patnai 23) in mds. per acre

Burdwan Farm Treatment	1951 Rainfall 29.6 inches	1952 Rainfall 25.1 inches	1953 Rainfall 35.9 inches
	Grain	Grain	Grain
No manure	21.70	16.00	28.52
Manure	34.29	24.17	42.45

From the accumulated mass of published results (Dept. Agric. Ann. Rep. 1924–42) of replicated unmanured varietal trials conducted on a group of si: heavy-yielding late-ripening varieties of rice at more than ten different experi mental stations located at different soil-climatic regions of the State, the dat

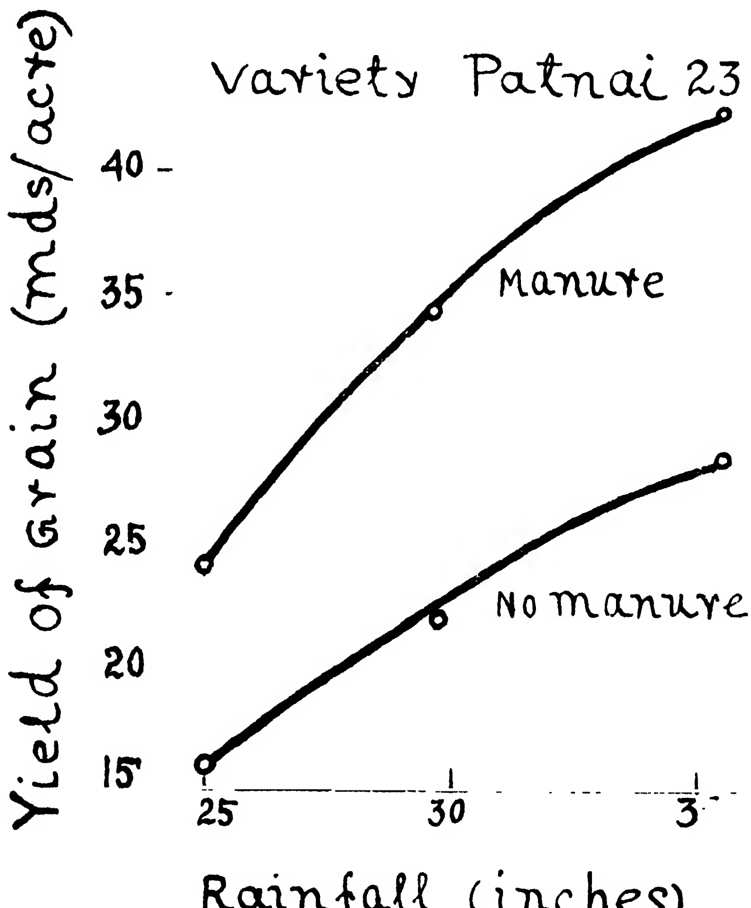

FIG. 1

of variety-wise grain yield along with the correspondng data of rainfall feeding the crop from transplantation to harvesting were compiled, classified and

processed. The data of rainfall and grain yield were tabulated *in seriatim* in the ascending order of magnitude of rainfall and the average was drawn up of rainfall at different domains and of the corresponding grain yield within that domain (Table II). Positive correlation was found between yield and rainfall. Considering

TABLE II

Average yield of rice and amount of rainfall

Variety of rice	No. of experiments	Average grain yield (mds./acre)	Average rainfall (inches) From transplantation to harvesting	Average rainfall (inches) During 30 days before transplantation
Bhasamanik	6	14.6	10.6	9.4
	14	22.1	20.2	11.5
	13	24.3	30.3	16.4
	8	25.2	38.6	19.0
	1	25.3	62.8	32.7
Dudsar	6	14.6	10.5	9.9
	8	21.6	20.7	11.8
	14	24.0	30.3	16.6
	9	24.2	38.3	19.0
	2	25.8	62.6	36.4
Jhingasail	6	13.8	10.6	9.5
	12	21.8	20.1	12.8
	6	22.8	30.1	16.1
	7	22.0	36.8	19.7
	1	25.6	62.8	32.7
Latisail	4	18.3	10.7	11.1
	6	23.8	21.3	13.0
	13	25.0	30.2	17.0
	8	25.0	36.4	16.0
	3	25.7	50.3	28.0
Indrasail	5	15.0	9.4	8.9
	5	23.1	19.8	12.2
	17	23.9	30.6	15.4
	4	24.0	42.0	21.5
	1	26.6	62.8	32.7
Nagra	1	13.1	22.9	6.2
	1	24.4	26.8	17.8
	5	27.3	30.3	15.1
	4	27.3	46.1	22.7

Correlation coefficient between average yield and average rainfall = +0.72

all the 29 values in the Table, the correlation coefficient between average grain yield and average rainfall worked out at +0.72. From 18 values where the rainfall total was within the domain of 30 inches, the corrleation coefficient came out as +0.82, which was highly significant. In case of the remaining 11 values, where rainfall exceeded 30 inches, the correlation coefficient was only +0.54, which was not significant even at 5% level. Inclusion of the values relating to higher ranges of rainfall over 30 inches had thus reduced the correlation coefficient from +0.82 to +0.72.

Data of average rainfall at different domains and corresponding average grain yield were plotted against each other and a set of six yield-rainfall curves was obtained for six varieties of rice (Figs. 2 to 7). Yield-rainfall curves showed that increasing amount of rainfall from ten to twenty inches induced a progressive and almost proportional increase in the yield of grain; with higher rainfall over twenty inches the increase fell off progressively till at the level of about thirty to thirty-two inches, the curves were flattened off and continued to do so till the highest level of sixty-three inches of rainfall was obtained. Any additional rainfall over thirty to thirty-two inches in the growing season did

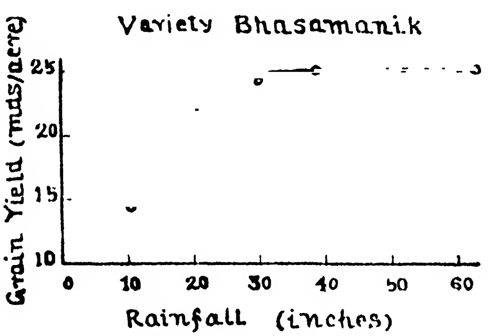

Fig 2

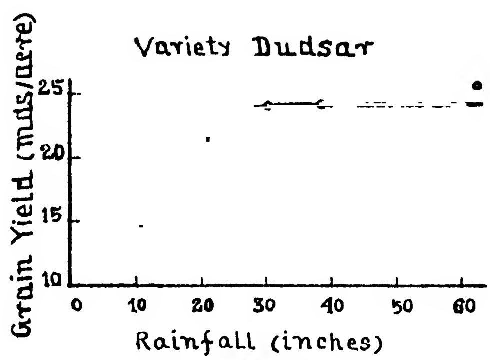

Fig 3

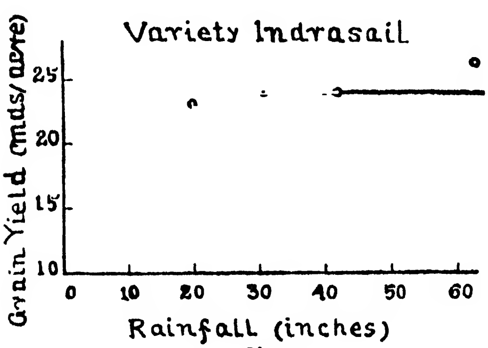

Fig 4

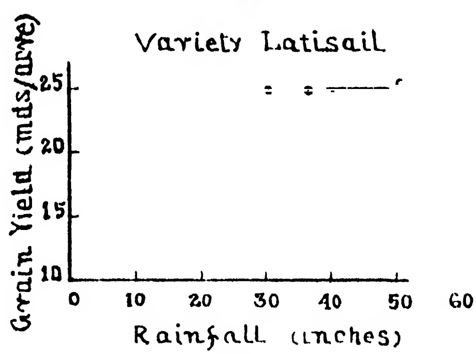

Fig 5

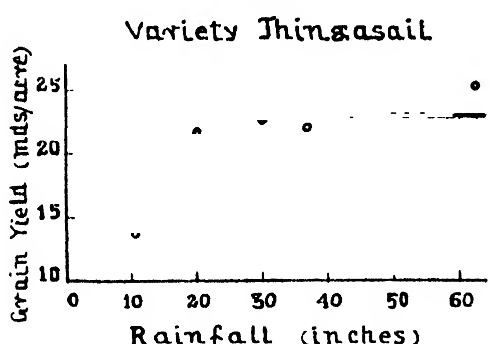

Fig 6

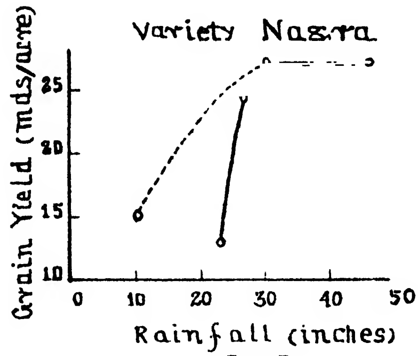

Fig 7

not exhibit any additional effect on yield. The minimum amount of rainfall required to produce optimum grain yield in different varieties of rice was thus calculated from the yield-rainfall curves.

Variety of rice	Minimum amount of rainfall inducing optimum grain yield (inches)	Grain yield (mds./acre)
Bhasamanik	32	25.0
Dudsar	30	24.0
Indrasail	29	24.0
Latisail	28	25.0
Jhingasail	30	23.0
Nagra	31	27.0
Average for varieties	30	24.7

It was significant to observe that at the lower domain of rainfall (below 30 inches), yield-rainfall curves of rice exhibited a similar behaviour to yield-water curves of upland crops but at the higher domain of rainfall, a completely different one. In the case of upland crops under limiting supply of water, progressive increase in water application tended to increase production of dry matter and yield of crops until a maximum was reached after which the effect was completely reversed. A seven-year average yield-water curve for wheat at Gooding, Idaho and a ten-year average yield-water curve for corn at Logan, Utah, (USDA Bulletin no. 1340) showed that increasing water application increased the yield of grain, although progressively slowly, till the maximum yield was obtained for wheat at 2 acre-feet and for corn at 3.25 acre-feet after which yield declined progressively sharply. Such a behaviour was also observed for some other upland crops (Roe, 1950). But the yield of rice, after attainment of a maximum, remained practically unaffected under increasing level of water supply, as the yield-rainfall curves would show. Indeed the rice crop could grow and flourish under moist, water-logged and even heavily submerged conditions. The success of cultivation of deep-water varieties in certain low-lying areas in India (West Bengal and Assam), East Pakistan, Burma, Thailand, Cambodia and Cochin China, where the plants in their early period of growth could withstand a rising column of flood water anything up to a level of twenty feet above field surface and even survive temporal over-flooding (Grist, 1953). was a testimony to the water tolerance capacity of the rice crop. This characteristic water tolerance capacity, rare in field crops, was probably one of the overriding reasons for its widest adaptations to the earth's surface to provide staple food for over half of its inhabitants.

Effect of Supplemental Irrigation on Yield

Earlier attempt made by the workers in this Department (Chakravorty, 1937–42) to find out the effect of supplemental irrigation on rice yield was not rewarded by any positive conclusions. Two typical transplanted water-logged varieties of rice (Bhasamanik and Patnai 23) were grown in well-bunded replicated plots under controlled conditions, one set of plots receiving normal rainfall and the other set an additional amount of irrigation water applied at an interval of a fortnight. Data of average grain yield and rainfall are given in Table III. The results showed that supplemental irrigation apparently had no beneficial effect on yield over mormal rainfall. But the minimum amount of rainfall feeding the crop during any growing season was as high as 30.96 inches and it was evident that this amount of rainfall was adequate to meet the water

requirement of rice so that a higher dose of rainfall or irrigation water had no influence on yield.

TABLE III

Effect of supplemental irrigation on rice cultivation
(Rice Research Station, Chinsurah, West Bengal)

Year	Variety	Average yield of grain (mds. per acre)		Average rainfall (inches) during the growing period	
		No irrigation	Irrigation	No irrigation	Irrigation
1937-38	Bhasamanik	35.8	36.0	33.11	33.87
	Patnai 23	37.5	36.4	,,	,,
1939-40	Bhasamanik	21.6	31.9	40.07	53.95
	Patnai 23	26.7	30.4	,,	,,
1940-41	Bhasamanik	29.3	29.5	30.96	38.09
	Patnai 23	23.9	24.9	,,	,,
1941-42	Bhasamanik	24.7	21.6	39.74	43.26
	Patnai 23	23.8	23.1	,,	,,
Average of 8 experiments		27.91	29.26		

WATER REQUIREMENT IN RELATION TO LOSS OF MOISTURE FROM RICE FIELD

Estimation of moisture losses from rice fields during the cropping season was attempted at elucidation of water requirement and soil-plant-water relations.

LOSS OF MOISTURE BY TRANSPIRATION

In America, Briggs and Shantz (1914) calculated the average transpiration ratio of rice as 519 in 1912 and 744 in 1913. In India, the average transpiration ratio was found to be of similar order of magnitude (Hector, 1925–28; Singh *et al.*, 1935). Recent determinations of transpiration ratio of the same and allied varieties of rice under comparable soil-climatic conditions gave an average of 626.6 (Ganguli, 1950). From the transpiration ratio, average optimum grain yield (24.7 mds. per acre) and straw grain ratio of 1.5, transpirational loss of water could be calculated as below :

Cured weight	*Dry weight*	
Grain—2038 lb	2038(1.00 - 0.15)	= 1732 lb
Straw 3057 lb	3057(1.00—0.20)	= 2446 lb
	Total	= 4178 lb

Roots at $\frac{4178}{11}$ = 380 lb

Total dry matter produced per acre = 4558 lb

The equivalent of the transpiration ratio of water of depth over 1 acre

$$= \frac{4558 \times 626.6 \times 12}{43{,}560 \times 62.5} = 12.56 \text{ inches.}$$

Loss of water by evaporation

Information is not available regarding evaporational loss of moisture from waterlogged rice fields during the growing season. Fundamental equations have been developed recently (Penman, 1948), which would seem to subscribe to the particular sets of climatic and field conditions as were normally associated with cultivation of transplanted rice. Application of the micro-climatic data, under collection in this Department for the last few years, to the Penman equation given below would render possible the calculation of the evaporational loss.

$E = 0{\cdot}35\ (e_s - e_d)\ (1 + 0.0098\ u_2)$ mm. per day, where

E is the rate of evaporation,
e_s is the vapour pressure of the water at the water surface,
e_d is the vapour pressure in the bulk of air at the dew-point and
u_2 is the speed of the wind 2 metres above ground-level in miles per day.

Five-year average of micro-climatic data, collected on rice fields at the State Rice Research Station at Chinsurah under this Department, along with monthly rates of evaporation from waterlogged rice fields calculated from the above equation and observed rate of evaporation from free water surface are given in Table IV. Monthly rates of evaporational loss from rice fields during the growing season of rice are summarised below

TABLE IV

Evaporation of water from waterlogged rice fields during crop growth
(Average of five years 1951–55)

Month	August	September	October	November	December
Mean temp. (°F)	83.0	83.8	80.9	71.3	65.5
Mean humidity (%)	86.6	87.2	87.4	73.0	69.4
Vapour pressure of water at 2 ft. above water surface in rice field (mm.)	25.3	25.7	25.5	15.3	12,3
Vapour pressure in the bulk of air at dew point (mm.)	21.6	22.5	20.6	10,5	7.8
Mean velocity of wind at 10 ft. above rice field (miles/hour)	3.72	3.09	2.26	1.57	1.58
Monthly evaporation from rice field (equation) (inches)	3.01	2.29	3.23	2.80	2.77
Monthly evaporation from free water surface (inches)	3.94	3.92	4.12	5.66	6.22

Month	Evaporational loss of water from waterlogged rice fields (inches)	Evaporational loss of water from free water surface (inches)
August	3.01	3.94
September	2.29	3.92
October	3.23	4.12
November	2.80	5.66
December	2.77	6.22
	14.10	23.86

Since the average date of transplantation was Aug. 10 and that of harvesting Dec. 16 (Table V), the evaporational loss during the growing period was calculated as 11.88 inches.

Loss of water by percolation

The problem of percolation, which depended on texture, structure and chemical composition of soil and sub-soil, depth to water-table, hydrostatic pressure of soil moisture and surface gradient, was further complicated in waterlogged rice fields through formation of compact cluster structure brought about by puddling of soils in soft and wet conditions under pressure. Puddling tended to make soils impervious though, hydrostatic pressure of standing water and possible movement of soil animals through soil profile could admit of slow percolation. But there was no precise information about the rate of such percolation. Indication was, however, obtainable from the balance-sheet of monthly rainfall and of corresponding moisture losses from rice fields. In addition to rainfall feeding the crop from transplantation to harvesting, an additional amount of moisture was available from puddled soil representing the difference between its moisture content at transplanting and harvesting times. Soils could hardly be puddled, unless the moisture content attained the *saturation capacity*, whereas the moisture content of soils at the time of harvesting of crop was sometimes above but usually below the *field capacity*, as was evidenced by the phenomenon of development of cracks. According to Kramer (1949), the moisture content of clay loam soils at field capacity was about 43 per cent and at saturation capacity 73 per cent of soils on dry weight basis. Hence

Depth of water per square inch in 12-inch layer of top soil at field capacity $\dfrac{12\times 85}{62.5}\times\dfrac{43}{100} = 7.01$ inches

Depth of water per square inch in 12-inch layer of top soil at saturation capacity $\dfrac{12\times 85}{62.5}\times\dfrac{73}{100} = 11.91$ inches

Additional amount of water available from puddled soils thus worked out at 4.90 acre-inches and total minimum water requirement at 34.90 acre-inches. Deducting the transpirational and evaporational losses, the loss by percolation worked out at 10.46 acre-inches.

Effect of Distribution of Rainfall on Yield

The value of rainfall to agriculture depends as much and often more on its distribution as on its absolute amount. Even under a given rainfall total in

TABLE V

Monthly distribution of rainfall

Level of rainfall (inches)	No. of experiments	Average date of			Average rainfall (inches)						Average yield of grain (mds/acre)
		Planting	Harvesting	Flowering	July	Aug	Sept.	Oct.	Nov.	Dec.	
10.23	26	Aug. 26	Dec. 15	Oct. 18	—	1.80	7.30	1.02	0.11	Nil	15.1
20.39	28	Aug. 14	Dec. 12	Oct. 25	—	9.62	7.51	3.25	Nil	0.01	22.0
30.08	53	Aug. 10	Dec. 16	Oct. 28	—	9.03	12.21	7.72	1.06	0.06	24.7
30.07	25	Aug. 11	Dec. 18	Oct. 31	—	7.66	11.14	9.29	1.90	0.08	31.5
Selected pattern)											
40.39	30	July 27	Dec. 18	Oct. 20	3.29	16.40	13.35	7.30	0.02	0.03	24.7
62-93	7	Aug. 4	Dec. 19	Oct. 25	—	28.32	20.88	13.43]	0.30	Nil	24.7

any growing season, the pattern of distribution that serves more closely the physiological water need of the crop at the different stages of its growth and maturity ovbiously gives a better crop response. Determination of the distribution pattern that induced optimum crop production was, therefore, important for assessment of water requirement at different phases of crop growth and ultimate regulation of the dose and timing of water application in any irrigational practice. It was also likely to give an indication of the extent of conservation or run-off under similar and different ranges of rainfall totals. For this purpose, the results were picked up of all those experiments where total rainfall feeding the crop during the growing season was in the neighbourhood of 30 inches corresponding to the point of inflexion of the yield-rainfall curves, monthly rainfall distribution figures were tabulated and an average was drawn up (Table V). Whether this pattern of distribution or any other was more conducive to yield was a matter of considerable importance. Plotting of grain yield against monthly rainfall and selection of distribution patterns recording some of the highest individual yields revealed that the selected distribution pattern induced about 27.5 per cent higher grain yield than the average distribution pattern of 30 inches or other ranges of rainfall totals (Table V).

Balance-sheets of loss and gain of water by rice fields in different months of the growing season under different ranges of rainfall totals have been drawn up on the basis of monthly rainfall and corresponding monthly losses of water (Table VI), and the resulting levels of soil moisture including depth of standing water on rice field have been graphically represented in Fig. 8 in order to bring out their comparative effect on physiological behaviour of rice in relation to yield. From Table VI, it would appear that under the selected distribution pattern (pattern 2), the soil was a little above puddled stage in August and there was a standing depth of about 2–3 inches of water in September and about 4 inches in October. Under distribution pattern 1 of same rainfall total, plants endured a deeper submergence of 5 to 7 inches in September and more than 7 inches in October corresponding to the period of active tillering. That the moisture conditions prevailing in rice fields under pattern 2 were suitable for optimum tiller formation had been demonstrated by Ghosh (1954) and other workers of this Department (Annual Report, 1925–26) and elsewhere in India (Sen, 1937; Singh *et. al.*, 1935), who had also shown that heavier depth of standing water at earlier stages of growth (7–8 weeks) over the puddled stage progressively suppressed tiller formation. Not only the number of tillers but also total leaf area, total dry matter and grain yield, which were highest under water at soil level, gradually decreased with rise of water level. Plant height and water content of both stem and leaf were also found to increase with rise in water level (Ghosh, 1954), thereby rendering the plants more susceptible to lodging. Deeper water level also adversely affected meristematic activity at regions of tiller formation and efficiency of utilisation of absorbed nitrogen in production of dry matter. Choudhuri and Ganguli (1948) found that 3–4 inches submergence during the growing period was best for tiller formation and grain yield. In Japan, the standard depth of water on rice field during the growing period was reported to vary from 1–2 inches (Grist, 1953).

The physiological rôle of standing depth of water and requirement of soil moisture during subsequent stages of growth relating to earing, flowering and and fruiting was yet little understood. It was, however, shown by Chakladar (1946), working on the influence of soil moisture on rice yield, that none of the plants under 33 per cent saturation formed seeds though some of them had flowered, while plants under 50–75 per cent saturation formed seeds. In both the distribution patterns of 30 inches rainfall, there was standing water till the middle of November. On the whole, 125 per cent saturation was maintained in soil under rainfall distri-

TABLE VI

Balance sheet of monthly rainfall and moisture losses from rice field

Level of rainfall (inches)	Month	Rainfall (inches)	Loss of moisture (inches) from rice field: Transpiration	Loss of moisture (inches) from rice field: Evaporation	Loss of moisture (inches) from rice field: Percolation	Total loss of moisture (inches)	Level of standing water/soil moisture at the end of the month (inches)	Average soil moisture during the month (% saturation)
30.08 (General pattern) Pattern 1	Aug. (22 days)	9.03	2.14	2.13	1.79	6.06	- 2.97	112
	Sept.	12.21	2.92	2.29	2.43	7.64	7.54	144
	Oct.	7.72	3.02	3.23	2.51	8.76	+ 6.50	159
	Nov.	1.06	2.92	2.80	2.43	8.15	−0.59	125
	Dec. (16 days)	0.06	1.56	1.43	1.30	4.29	−4.82	77
30.07 (Selected pattern) Pattern 2	Aug. (21 days)	7.66	2.58	2.04	1.71	6.33	1.33	105
	Sept.	11.14	3.69	2.29	2.43	8.41	- 4.06	123
	Oct.	9.29	3.82	3.23	2.51	9.56	+3.79	133
	Nov.	1.90	3.69	2.80	2.43	8.92	- 3.23	102
	Dec. (18 days)	0.08	2.22	0.54	0.48	3.24	−6.39	60
10.23	Aug. (6 days)	1.80	0.41	0.58	0.49	1.48	− 0.32	101
	Sept.	7.30	2.05	2.29	2.43	6.77	0.85	105
	Oct.	1.02	2,11	2.77	2.02	6.90	- 5.03	83
	Nov.	0.11	2.05	0.84	--	2.89	-7.81	46
	Dec. (15 days)	Nil	1.02	0.01	—	1.03	8.84	30
20.39	Aug. (18 days)	9.62	1.66	1.75	1.46	4.87	4.75	120
	Sept.	7.51	2.77	2.29	2.43	7.49	- 4.77	140
	Oct.	3.25	2.87	3.23	2.51	8.61	-0.59	118
	Nov.	Nil	2.77	2.01	1.45	6.23	−6.82	69
	Dec. (12 days)	0.01	1.11	0.01	—	1.12	−7.93	38
							Potential level of standing water (inches)	Level of standing water/soil moisture after allowing runoff losses over 10″ on field level (inches)
40.39	July (5 days)	3.29	0.44	0.55	0.41	1.40	− 1.89	+ 1.89
	Aug.	16.40	2.68	3.01	2.52	8.21	10.08	- 10.00
	Sept.	13.35	2.60	2.29	2.43	7.32	- 16.11	10.00
	Oct.	7.30	2.68	3.23	2.51	8.42	− 14.99	5.98
	Nov.	0.02	2.60	2.80	2.43	7.83	− 7.18	− 1.17
	Dec. (18 days)	0.03	1.56	1.61	1.44	4.61	− 2.60	− 3.41
62.93	Aug. (28 days)	28.32	2.55	2.72	2.28	7.55	- 20.77	− 10.00
	Sept.	20.88	2.73	2.29	2.43	7.45	- 34.20	− 10.00
	Oct.	13.43	2.82	3.23	2.51	8.56	− 39.07	- 10.00
	Nov.	0.30	2.73	2.80	2.43	7.96	− 31.41	− 2.34
	Dec. (19 days)	Nil	1.73	1.70	1.54	4.97	− 26.44	− 2.63

bution pattern 1, and 102 per cent saturation under pattern 2 during the month of November.

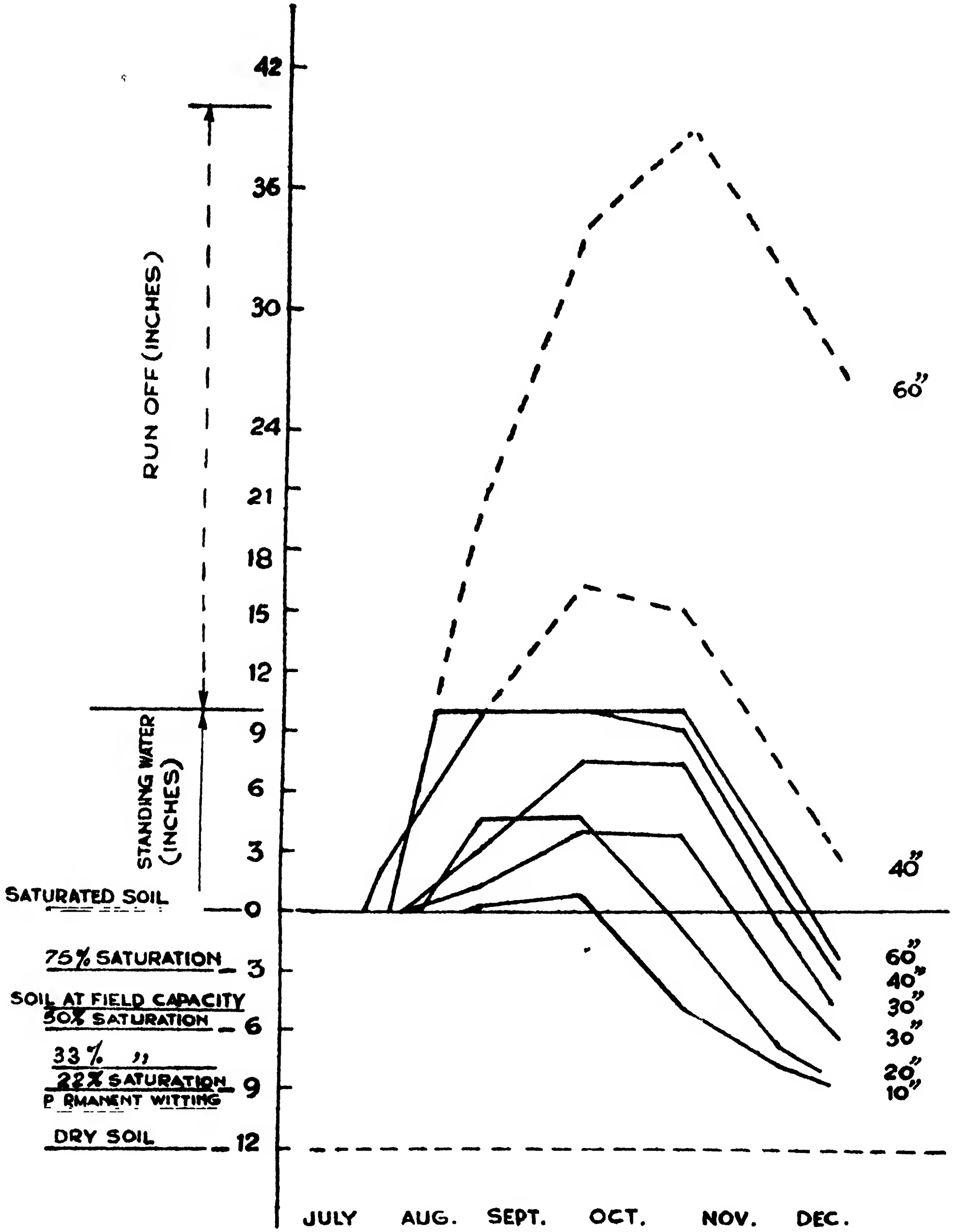

FIG-8

At the level of 10 inches rainfall, the field was just at puddled stage in August and September but standing water was dried up in early October before emergence of ears took place. Soil moisture was reduced to 83 per cent saturation

on average in October and to 46 per cent saturation in Nobemver. Moisture condition in August and September was not quite unsuitable for tillering, though available period for tillering was shorter due to slightly delayed transplantation (August 26) caused by inadequacy of preceding rainfall. Delayed transplantation might affect yield (Dept. Agric. Anna. Rept, 1934–39; Ganguli, 1950), though the main reason for abnormally low yield would seem to be inadequate earing coupled with under-development of seeds due to grave deficiency of soil moisture. At the level of 20 inches rainfall, there was suitable depth of standing water in August and September for adequate tillering but at the fruiting stage (Nov.), soil moisture was reduced to 69 per cent saturation. It would seem to show that 69 per cent saturation during fruiting stage was not sufficient for optimum grain formation. At the level of 40 and 63 inches rainfall, there was plenty of moisture in the field at all stages of growth and development for adequate tiller and grain formation. But heavier rainfall was likely to result in so much accumulation of water on well-bunded rice field as to cause proportionate run off.

It was thus evident that maintenance of certain specific levels of standing water and soil moisture in rice fields at different stages of crop growth and maturity was essential for optimum tillering and grain formation in a transplanted rice crop. Such specific levels of soil moisture could scarcely be maintained under field conditions except through controlled irrigation and drainage. The overriding influence of water control on the success of rice cultivation was sought to be brought out in course of technical discussions on soil-plant-water relations at the Fifth session of the International Rice Commission in Calcutta in November, 1956. Evidence was adduced that countries having controlled systems of water supply consistently obtained higher outturns of rice than those growing the crop otherwise under rain-fed conditions and instances were cited that water control in Japan and Italy was primarily responsible for higher rice yields than other countries of Asia including South East Asia and the world.

Effect of Frequency and Depth of Rainfall on Yield

Average frequency of rainy days during different months in the growing season and depth of showers per rainy day at 30 inches of rainfall total have been calculated in Table VII.

TABLE VII

Frequency of rainy days and depth of shower (30 inches rainfall)

Month	Average number of rainy days per month	Average depth of water per rainy day (inches)
August	20	0·70
September	16	0·75
October	7	1·02
November	1·5	0·70

Frequency and depth of precipitation (Table VII) at 30 inches rainfall did not indicate run-off losses in view of lower level of standing water on rice fields (Table VI and Fig. 8) in relation to height of protective bunds, though higher rainfall ranges and even concentrated downpour on any or closely successive

occasions could cause it. The estimated requirement of 30 inches rainfall would seem to secure maximum possible conservation and utilisation of received rainfall.

The pattern of frequency and depth of precipitation in different months of the growing season would seem to have important physiological bearing on rice plants particularly relating to yield. Frequent light showers in August and September helped in cooling the atmosphere of the hot summer and in further lowering of temperature of leaf and stem surface through evaporation of intercepted rains. Lower temperatures and higher humidity both contributed to reduction of intensity of transpiration and oxidative catabolic processes leading to more rapid fixation of dry matter: they also reduced evaporational losses thereby effecting overall economy in water utilistion. On the other hand, less frequent showers of heavier depth in October caused less dissipation of moisture in the atmosphere and more accumulation in the field, which was helpful in carrying the crop through the "critical stages" of flowering and fruiting. Besides, higher frequency of rainfall had a deleterious effect on flowering and fertilisation. Choudhuri and Ganguli (1948) observed that while flowering of rice was at its maximum between 10 to 11 A.M. and was finished before midday in sunny days, in rainy days and even in cloudy weather flowering began late and continued upto 3 to 4 P.M. The flowers opening in the afternoon were likely to do so with their anthers already dried up (Burkill as quoted by Grist, 1953), thereby affecting the chance of enclosed pollens of bursting out and fertilisation. High altitude angular bombardment of rain drops on opening flowers and also accompanying wind could cause wasteful shedding of anthers in plenty. These reasons, therefore, reflected the advantage of wider interval and perhaps complete cessation of rains during the period of flowering for promotion of yield.

Water requirement for Raising Seedlings and Pre-transplantation Agricultural Operations

Thirty days old seedlings were considered to be best for transplantation in normal times (Dept. Agric. Ann. Rep. 1934–39: Choudhuri and Ganguli, 1948; Roe, 1950), and hence water requirement for raising such seedlings was calculated. It was known that seedlings would grow well in a soil at or above field capacity and that they would not grow at all unless the soil moisture was above the limit of permanent wilting. From the moisture content of clay loam soils at field capacity and at permanent wilting range and from the rate of evaporational and transpirational losses of moisture during 30 days of growth of seedlings, minimum and adequate water requirement was calculated as in Table VII.

The results (Table IX) of field experiments on raising of 30 days old seedlings to suitable heights for transplantation (Dept. Agric. Ann. Rep., 1933), showed that the amount of rainfall (10·8 inches) received by these seedlings closely reconciled with the magnitude of adequate water requirement calculated in Table VIII (10·74 inches). Adding up water requirement (4·90 acre-inches) for puddling of a clay loam soil, already at field capacity, up to a depth of 12 inches representing the puddling zone and the potential rhizosphere, the total water requirement for the entire process of raising seedlings and conducting tillage and other pre-transplantation agricultural operations including puddling of soils was calculated at 15·64 acre-inches.

Water requirements for the above processes were also found out from the field data by a different method. The amount of rainfall recorded during 30 days of pre-transplantation period in individual experiments (Table II) were plotted against the corresponding number of rainy days. The majority of points representing about two-thirds of the total number were found to cluster within the

TABLE VIII

Water requirement for raising seedlings

Nature of water requirement	Amount of water requirement (inches)	
	Minimum	Adequate
Water required to bring the twelve-inch layer of surface soil to moisture level of	Permanent wilting point $\left(12 \times 1{\cdot}36 \times \frac{16}{100}\right)$ 2·61	Field capacity $\left(12 \times 1{\cdot}36 \times \frac{43}{100}\right)$ 7·02
Water required to recompense evaporational loss in 30 days in July (Penman equation)	3·41	3·41
Water required to recompense transpirational loss in 30 days	0·31	0·31
Total	6·33	10·74

TABLE IX

Rainfall for raising seedlings (Experimental Farm—Pabna, 1933)

Variety	Date of sowing in seed-bed	Quantity of Niciphos added/acre	Age of seedlings (days)	Height of seedlings (inches)	Average rainfall received (inches)
Indrasail	14-6-32	20 lb. N 20 lb. P_2O_5	31	16.0	11.2
,,	,,	Nil	34	13.5	12.3
Dudsar	3-7-32	20 lb. N 20 lb. P_2O_5	24	15.2	8.8
,,	,,	Nil	29	13.5	10.6
Chinsurah II (Bhasamanik)	13-7-32	20 lb. N 20 lb. P_2O_5	29	13.0	10.0
,,	,,	Nil	35	10.5	11.9
		Average	30	13.6	10.8

orbit of a very small circle (Fig. 9) and the average rainfall calculated from these clustered points was found to be 12.9 inches. Adding the moisture content of field soils, which had never been completely dry but retained moisture

not usually below the wilting range prior to this period, water requirement worked out as 15°.5 acre-inches.

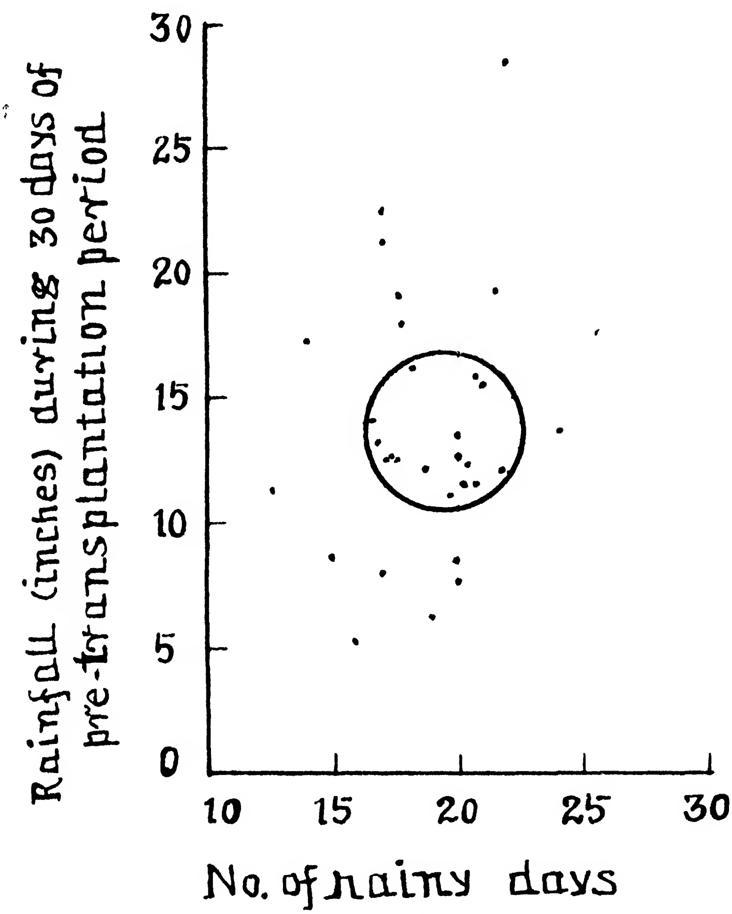

Fig. 9

Rôle of Rainfall on Nitrogen Supply

Rain water contains nitrogen, mainly as ammonium nitrate and nitrite and partly as free ammonia (Partington, 1953), varying from 1 to 2 parts per million parts. It is estimated that 30 inches rainfall can supply about 10 lb. of nitrogen per acre. In terms of 20 lb rice grain obtainable per lb. of nitrogen applied in the form of manures and fertilisers at the level of 30 lb. N_2 per acre (Basak *et. al.*, 1957), 10 lb N_2 of rain-water can, therefore, contribute to production of at least 200 lb (2.5 mds.) of additional grain per acre and probably a little more in accordance with the law of diminishing returns.

The supply of ammonium nitrogen in any downpour was obviously insignificant in comparison to its supply from waterlogged rice soils. The concentration of ammonium nitrogen, which was practically nil at flooding time, rose to 1.25 mg. per 100 gm. dry soil after 20 days of submergence and increased slowly to the higher concentration of 2.5–3.0 mg/ 100 gm. dry soil with prolongation of submergence (Basak, unpublished results). Thus in addition to maintaining supply of ammonium nitrogen requirement of growing rice crop, 2 million lb. of

top soil contained 25 lb. of ammonium nitrogen per acre after 20 days of submergence and higher amounts of 50–60 lb on prolonged submergence. Hence contribution of any individual shower of rains to reflect any change on NH_4–N concentration of soil in the rhizosphere was likely to be negligible. Under the circumstances the assumption by rice plants of characteristic liveliness and vigour coupled with deepening of colour closely following a shower would seem to indicate that nitrogen of rain water was probably directly absorbed and quickly assimilated by the intercepting leaves and growing parts of the plants as were nearest to the *foci* of active systhesis and rapid deposition of dry matter. Recent work on plant metabolism with suitable and radioactive isotopes has already shown the occurrence of such direct absorption of nutritional elements (N^{15}, P^{32} and K^{42}) by the leaves and their subsequent migration to and utilisation in the requisite parts of plants (Thorne, 1955). Since only 35–40 per cent of nitrogen applied to water-logged soils was recoverable by a rice crop (Mitsui, 1955; De and Digar, 1955; Basak *et al.*, 1957), such direct absorption of nitrogenous nutrients was indicative of high level of efficiency and economy in assimilation and utilisation of applied nitrogen than feeding it in bulk through the soil.

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Summary

Attempt was made at determination of water requirement of transplanted rice from the effect of rainfall on yield. Significant positive correlations were found between average grain yield and average rainfall. The correlation coefficient was highly significant upto 30 inches rainfall but at higher ranges of rainfall total, it was not significant. Similarly, the yield-rainfall curves showed that increasing amounts of rainfall over 10 inches induced a progressive increase in grain yield till the optimum yield was attained at 30 inches rainfall total. Thereafter, any additional rainfall did not produce any additional effect on yield. It was concluded that water requirement of rice from transplantation to harvesting was of the order of 30 acre-inches. Results of supplemental irrigation confirmed these conclusions.

Loss of moisture from water-logged rice fields was determined for elucidation of the physical and physiological basis of water requirement. Loss of moisture by transpiration was calculated at 12.56 inches, by evaporation during the growing period from transplantaton to harvesting at 11.88 inches and by percolation at the level of 30 inches rainfall during the same period at 10.46 inches.

Patterns of monthly distribution of rainfall, during the growng period of rice, under different ranges of rainfall total were studied to find out the phased water requirement at different stages of crop growth. The ideal pattern of rainfall distribution that served the phased water requirement for production of optimum yield was determined as follows:

August (21 days)	7.66 inches
September	11.14 ,,
October	9.29 ,,
November	1.90 ,,
December (18 days)	0.08 ,,

Balance sheets were drawn up of monthly rainfall and corresponding monthly losses of moisture by transpiration, evaporation, percolation and run-off so as to evaluate the resulting levels of soil moisture and standing water on rice fields during the different stages of crop growth and maturity and their relative influence on yield. The ideal level of soil moisture

and standing water that contributed to optimum tillering and grain formation has been given below and the importance of water control on success of rice cultivation has been discussed.

August ..	1 to 2 inches of standing water
September ..	2 to 3 „ „ „
October ..	3 to 4 „ „ „
November ..	100 per cent saturation of soil moisture
December .	50 „ „ „

Frequency and depth of rainfall in different months of the growing season and their physical influence on temperature control and water economy and physiological significance on plant metabolism and crop yield were studied. Frequent light showers during the earlier stages of growth from tillering to ear emergence and less frequent but heavier depth of showers at subsequent stages were found suitable for promotion of yield. The effect of rainfall on flowering and fertilisation has been discussed.

Water requirement for raising 30 days old seedlings and for tillage and other agricultural operations including puddling of soils has been found to be 15.5 acre-inches. Thus the total water requirement for raising a good crop of transplanted waterlogged rice from the time of sowing of seeds to harvesting of the crop has been calculated as 45.5 acre-inches.

Contribution of rainfall to supply of nitrogen in the nutrition of rice crop and the probable mechanism and efficiency of its absorption and assimilation have been elucidated.

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SANTAL SIBS—THEIR TRADITIONAL HISTORY AND OBSERVED INTERMARRIAGE FREQUENCIES

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The Santals are organised on the basis of exogamous sibs. The sibs are termed *jati* which in Hindu Society denotes caste. The traditions transmitted from one generation to another and narrated at certain ceremonials, state that in the beginning a man and a woman were born from two eggs of a pair of geese. They are old man Pilchu (Pilchu Haṛam) and old woman Pilchu (Pilchu Buḍhi), the original ancestors of the Santals. This ancestral pair had seven sons and seven daughters. In course of time each son married one daughter and from these seven married couples originated the seven original Santal sibs—Hasdak, Murmu, Kisku, Hembrom, Marandi, Soren, and Tudu. Five more sibs were later added. These are Baske, Besra, Pauria, Core and Bedea. According to Kolean, whose account was noted by Skrefsrud and edited by Bodding (1928) the last named group had disappeared when he reported these details.

In Santal Parganas and in Bengal, the writer found similar traditions prevalent, although in some points there were important differences. The tradition in Mayurbhanj differed in certain respects. The names of ten sibs other than Pauria and Bedea are identical in the two areas. The Pauria are also known, but it was stated in Mayurbhanj that they sometimes call themselves Copiar Murmu, without having any right to the term Murmu, or simply Copiar. The twelfth name of Bedea was not given in Mayurbhanj. In one account it was stated to be Gondwar. This sib was actually found in Mayurbhanj. In another account, the name was said to be Dorka and to refer to "Birhors and others" according to the informants. The reference was evidently to Santals who had intermarried with non-Santals like Birhors. In the Santal Parganas the writer carried out careful enquiries in a number of villages, enlisting the help of Santal school teachers and also of village elders who came to various fairs. Several families of "Bedea" Santals were eventually reported to be staying in a village off the main road in Jamtara area. These people claimed to be Santals. But the Santals of neighbouring hamlets stated that they were of mixed Santal descent. They were referred to as Bedea Santals and this was admitted by an agnatic kin of the family. The tradition noted in Mayurbhanj of intermixture for the twelfth sib fits in with what was actually found in Santal Paraganas and also with the report of disappearance of this group by Kolean (since the Bedeas did not report as Bedeas).

The Santal sibs are each divided into sub-sibs termed Parist. Originally, marriage between sub-sibs inside the sib was prohibited. In recent years there has been relaxation of this rule, especially in Mayurbhanj and in some parts of Santal Parganas. Nevertheless, such instances are rare. An examination of 1568 marriages selected in the Santal areas in Bengal on random sampling technique revealed (Chattopadhyay 1947) only 28 such marriages in the sib. In the Santal Parganas there were six such marriages out of 289 studies. This is barely two per cent. It was stated in the villages of Bengal by elders that the prohibited relatives —the Ban Ganok Perà—include all sib members besides certain other relatives. Marriages in the sib but in a different sub-sib were not proper. Thus a Hat Soren should not marry any other Soren such as Man Soren. But sometimes this is

done now-a-days and tolerated or a fine is imposed. One of the rules which regulate intermarriage in different groups is that the Abge and Orak' Bonga (house deities and deities of smaller social groups) of the two families must be different. These are ancestral Bongas of the Parist and family. It was found on survey that a boy and a girl with different Abge bongas and belonging to the same Parist had married in Silda area. There had been trouble over the matter but as it was in a big village with a large literate Santal population, and in an area subject to strong modern Hindu influences, the marriage had not been dissolved. These are indications of changes that are taking place.

In Mayurbhanj, marriages in sub-sibs of the same sib are common. Out of 155 marriages studied in Muruda area as many as 15 were within the sib, in different sub- ibs. The elders said this was not held to be wrong now.

In the traditional songs sung at Jomsim *binti*, there is reference to a period when sib exogamy was not practised and marriage with near kin seems to have occurred.

It has been found from a study of Santal villages that these are founded by a group of men from a single sib and that their descendants form the nucleus of the village. Detailed studies of origin and development of villages show that the growth of villages took place mainly on a single sib basis until others were invited to settle. Among the Mundas of similar speech and culture and traditionally of same stock living in Chotanagpore, it is stated by Roy (1912) that when a village became over-populated, leading families of the same sib went out and founded a new village. In this way a number of daughter villages grew up round that original village.

The Munda political structure centered thus round the sib and sib settlement. The main office bearers came from the founder's family and sib in each village and the head of the group of villages was furnished by the original founder's family in the central mother village.

It has been found that among the Santals also, the head man of a village comes from the founder's family and sib. Santal villages are not however single sib villages now. But there are clear traditions of single sib villages and domains at least in Chae Champa country (Hazaribagh plateau) and probably dating to an earlier period. Subsequently the Santals seemed to have migrated too frequently, and in territory where there were overlords, for the sib to have any political and territorial basis having the character of a State. The following extract from Risley (1892) is noted with regard to their traditions in Chae Champa :

"In order that members of the various septs may recognize each other when they meet, each sept (= sib. KPC), except Pauria, Chore and Bedea, has certain pass-words peculiar to itself, which are supposed to be the names of the original homes of the septs in Champa or in one of the earlier settlements of the tribe. The passwords are as follows :

(1) Hasdak—Tatijhari, Gangijauni, Kara Guja, Sohodoro; (2) Murmu—Champagarh, Bagsumbha, Naran Manjhi; (3) Kisku—Kundagarh; (4) Hembrom—Kunda, Khairigarh, Jalaghatia; (5) Marandi—Badoligarh, Jelen Sinjo, Dhano Manjhi; (6) Saren—Andali, Barha, Pero Pargana; (7) Tudu—Simgarh, Sukrikutup, Baru Manjhi; (8) Baske—Ranga, Chunukjhandu; (9) Besra—Dhokrapalania, Gulu, Phagu Manjhi."

The place names are easily identified in the Chae Champa area in Hazaribagh. There is an ancient tale in this connection told at Jomsim *binti* when other ancient traditional tales are recited. It speaks of a quarrel between the ancestors of Kiskus and Marandis. The story and songs connected with it clearly indicate separate territory of each of these sibs. Also each sib had its own headquarters. It was Koendagaṛ for the Kiskus and Badoligaṛh. (gaṛ = fort) for the Marandis. The story and songs are not noted here for lack of space. It is clear from these tales and songs that there was a dispute about the limits of territory of the Kiskus

of Koendagaṛ and Marandis of Badoligaṛh. The identification phrases noted by Risley mention these two forts for these two sibs, and also other areas for seven other sibs. It is therefore permissible to conclude that in the Chae Champa area, the Santals lived in territories demarcated for each sib, where villages were also single sib domains, as found much later by Roy among Mundas in their intact villages.[1]

There is another story of a minor disagreement, between Tudu and Besra, which did not affect the sib as a whole, nor did it lead to war. It was a quarrel over a Tudu boy coming to dance with Besra girls. The point of interest in the story is that according to it, the Tudu terrtory and Besra territory were separated by a river showing that there was demarcation. The quarrel did not lead to avoidance of the two sibs in marriage. There is a tradition that 'at first Besra girls were not married by other Santals. But a Tudu boy who danced with Besra girls, married one of them. Others followed suit. It was also stated that the Besra and Tudu originated the custom of giving three pieces of cloth at marriage, besides money. In the descriptive terms for sibs the Tudu are referred to as Mandariya i.e. drum players (at dance) and the Besra as Nacaniya i.e. girls who dance.

It is clear that the Tudu and Besra are linked together in tradition as (a) drummers and dancers, (b) as having originated, jointly, a marriage custom, and (c) as being the two sibs which intermarried first leading to admission of Besras into Santal society by marriage. It has been considered necessary to add these details to make it clear that the quarrel of Tudu and Besra did not lead to avoidance in marriage but ended in promotion of their union. On the other hand, the war between Kisku and Marandi had far-reaching consequences and the breach between them persisted. If marriage occurs between these two sibs, they are said to be unhappy. In connection with two cases of dissolution of Marandi-Kisku unions by divorce a generation earlier, in two genealogies collected by the writer, the relatives who were Kisku, at once made this comment. When other cases of divorce where husband and wife belong to other sibs came up for record, no such comment was made. The sample studied of Kisku-Marandi marriages and other marriages was not large enough for the comparison of relative frequency of divorces. But the data on marriages between different sibs furnish evidence of the dislike of unions between Kisku and Marandi sibs. For Tudu and Besra the frequency of marriages does not support any mutual avoidance of these two sibs in marriage. This is in agreement with the traditions noted.

The frequency calculations are made only for marriages in the Bengal Santal areas. The Santal villages in Bengal as noted earlier, were selected on a random sampling technique, in consultation with the Indian Statistical Institute. In Santal Parganas, the areas were (a) a sub-division subject to Bengalee influence, and (b) a small area in the Santal reservation. The villages were widely scattered and hence the study gives a fair picture of the areas. Nevertheless, in the absence of complete randomised samples, numerical figures cannot be used by themselves as indicators of existing rules, except in a broad general way. The size of the sample for Santal Parganas is fair, the total being 294 marriages. But the expected frequency of marriages of even the most numerous sib is less than 10. Sampling errors even when properly randomised are likely to give wide divergences in all cases where the expected frequency is less than 25. In Santal Parganas, the sample was not randomised on a scientific basis. This is true also for Mayurbhanj. Here there was another

[1] The conditions under which such conclusions may be drawn are discussed in the writer's paper (Chattopadhyay, 1942).

defect in noting the data. As the genealogies were recorded while observing rites and ceremonies and for enquiries connected with them, the sib of members of the genealogy noted in any instance was ascertained at first for the persons affected. In general, enquiries of sibs of marriage relations and deceased members have to be made in the family circle and the full information becomes available after enquiry from several individuals. In Mayurbhanj this subsequent enquiry was made only in a limited area. A fair proportion of genealogies collected while studying material culture and ceremonies in widely separated areas was left out of the later enquiries to ascertain sibs of all persons in the genealogy. The marriage pattern is also somewhat different. Hence, as the total number of marriages was less than 200, the detailed analysis of frequencies of sib relations has not been carried out in this case as well. Such analysis is not statistically justified. A brief indication of the lines of testing is given below.

Let N be the total number of marriages between different sibs excluding marriages in the sib. Let P, Q ... etc be the total number of marriages of the sibs P, Q ... etc. considering men only of such sibs who are marrying in other sibs. Let p, q ... etc be the total number of similar marriages of sibs P, Q ... etc considering women only of such sibs. The total of marriages of men will be the same for women, for all sibs. Then, if the frequency of intermarriage of sibs P and Q is independent of any bias towards or away from such marriage, the chances of such unions will be given by a simple formula which is the product of the ratio of the numbers of marriage partners in each sib to the total. For men of sib P and women of sib Q, the likelihood is $\frac{P}{N} \times \frac{q}{N} = \frac{Pq}{N \times N}$ and the expected frequency is this fraction of the total number N, that is $\frac{Pq}{N}$. Similarly for women of P and men of Q, the number will be $\frac{pQ}{N}$. The expected frequencies of marriage between different sibs may be calculated in this way. Separate calculations for men and women are necessary, as the number of marriages is not the same for union between any two sibs although the total for all sibs together is the same for men and women. Apart from any bias in marriage, there will be some difference between the two sets of values, due to sampling fluctuations.

Two points have to be remembered in this connection. If the expected frequency is low, say less than 10, then a difference of one or two marriages will cause a big divergence in percentage. Actually, if the expected frequency is low, and the population (here, the total of marriages of sib, separately for each sex) is below say 50, two small sibs may be combined in one unit, to bring the total in each case within the limits required. This is a recognised statistical device. As the total population of Besra men and of Besra women, as defined above, is in each case barely 30 and the expected frequencies are 5.5 and 4 only, the figures for Baske and Besra have been combined under "other sibs". There are no Pauria or Core cases in the Bengal list. In the Table for Marandi marriages below, the difference between expected frequency and actual frequency is shown to the nearest integer.

The value noted in the last row in symbols used by Yule is of $(\tilde{m}_r - m_r)^2/\tilde{m}_r$ where $\tilde{m}_r$ denotes the expected frequency and m_r the observed frequency in the rth cell. As there are in this Table seven sib groups in which the Marandi married (counting "other sibs" as one), and the total is given, one degree of freedom is subjected to constraint. There are thus six degrees of freedom. The value of 41.44 for chi squared, as the sum is called, gives a probability value for six

TABLE 1

Marandi marriages with other sibs

Sib	Murmu	Kisku	Hasdak	Hembrom	Soren	Tudu	Other Sibs	Total	Per cent of total difference
Frequency expected for Men	61.4	13.9	37.2	29.7	47.8	25.4	14	229.4	
Actual for Men	74	1	48	32	72	34	13	274	
Difference in integers	13	−13	11	2	24	9	−1	+45	19.6%
Expected for Women	41.2	10.8	28.2	29.6	29.4	26	10.6	175.8	
Actual for Women	53	8	25	45	33	38	12	214	
Difference in integers	12	−3	−3	15	4	12	1	+38	21.6%
Total of men plus total of of women expected	102.6	24.7	65.4	59.3	77.2	51.4	24.6	405.2	
Actual of men plus actual of women	127	9	73	77	105	72	25	488	
Difference in integers	24	−16	8	18	28	21	0	83	
Difference per cent of expected	23.3	−64	12.3	30.5	36.4	41.2	0	—	20%
$\frac{(\text{Actual Difference})^2}{\text{Expected Frequency}}$	5.59	10.24	0.98	5.49	10.19	8.95	0	41.44	

degrees of freedom, which is in this case infinitesimal. Hence the likelihood of the differences being due to sampling fluctuations is negligible.

The difference between expected and actual frequency is positive for all sibs except one serious (Kisku) and two minor exceptions and indicates a bias towards marriage in such sibs. For the "other sibs" there is no such bias. For the Kisku, there is clear antipathy to marriage with Marandi. The difference for men and women taken together is –64 per cent which is far beyond the limit of sampling fluctuation. If we exclude the Kisku marriages, the total comes to 479 for all marriages, against an expected value of 380 only. The difference is 99 which is 26 per cent of the expected value. This indicates a bias towards marriage in such sibs.

A more detailed analysis of the marriage frequencies is needed for ascertaining the likely factor causing this excess. It has been stated by some investigators among Santals, that the mother's sib is avoided by the Santal in marriage. When the present writer made this enquiry in the abstract, putting the question in a language other than Santali as well as in Santali, he was told that such marriages did not occur. When however he put the question in a concrete form by referring to the genealogies of the persons questioned and of other persons belonging to their mother's sib but not nearly related to them, the reply was that such marriages were in order. As the discrepancy was curious, and suggested some submerged belief against some kind of marriage with the mother's sib members, all the marriages studied, where the mother's sib is known and recorded were specially tabulated. The tabulation was to indicate the number of marriages of each sib with other sibs, separately for marriages in the mother's sib as well as marriages out of the mother's sib. The total of marriages of both types was 912 out of the 1540 marriages so far studied. Normally, in a simple family we can expect, of all the marriages tabulated about half to two thirds of cases where the information about the mother's sib will be available. For, in the top generation, beyond which the information has not been collected, this bit of data will always be absent. The proportion obtained with full data is, therefore, fair. The details are noted in Table II below.

TABLE II

Marriages in and out of mother's sib

Category	Number	Per cent of Total
In Mother's sib	217	23.8
Out of Mother's sib	695	76.2
Total in all sibs	912	100

It is not possible to determine the exact likelihood of marriage in the mother's sib without complex calculations and additional data. But on an average, without any bias we might expect about one seventh at most of marriages in the mother's sibs. The actual figures as noted in Table II indicate the existence of a very large bias in favour of marriage in the mother's sib. The reasons for such a bias, in the opinion of the writer, appears to be due to the former existence of a marriage custom which has now disappeared. One of the likely types of marriage which would, after its disappearance, leave behind a bias in favour of marriage in the mother's sib, is marriage with own mother's brother's daughter.

Since this form of marriage was once prevalent in North India, both in the time of the Mahabharata and in the lifetime of Buddha, and since it survives to the present day in Penninsular India, as well as on the border area of Eastern India, some support is given to such a hypothesis. The occurrence of this form of marriage among tribal folk to the west and south of Chotanagpore seems to strengthen such a view. There are however other alternatives and the question requires discussion in a separate paper. From the point of view of the women it may be said that women given in marriage to a family tend to be, in succeeding generations, of the same sib. The close connection of a man in rituals with his father's sister's husband, his own sister's husband and with the daughter's hasband, as also the sexual joking relationship found between a woman and her father's sister's husband fit in with this marriage trend, of which the significance has been revealed by statistical analysis.

Abstract

In this paper the writer notes a brief account of the traditional origin of Santal sibs and of the tradition of separate demarcated states of each sib. A statistical analysis is then carried out of 1540 Santal marriages to find out bias towards or away from marriage between different sibs. The tradition of Marandi-Kisku avoidance in marriage is tested and found to be correct. On the other hand, contrary to reports by some earlier observers, a definite bias in favour of marriage in the mother's sib by a man is proved by the figures of such marriages. A likely explanation is suggested.

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AN EXPRESSION FOR THE GROWTH-COEFFICIENT α IN THE LAW $y=bx^{\alpha}$ OF CONSTANT DIFFERENTIAL GROWTH RATIO, EXPRESSING THE GROWTH RELATIONSHIP BETWEEN THE BODY SIZE x AND THE ORGAN SIZE y, IN VARIOUS ORGANIC FORMS

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Introduction

Although almost all organic forms are the results of differential growth, this problem did not receive the notice of scientists till Julian S. Huxley (1932) and others, from their study on a vast number of species of animals, ventured to show an entirely new method of approach to its study. Huxley gave a detailed application of the quantitative expression to the general law of differential growth, to describe adequately a wide range of growth phenomena in animals and plants, in the form of the formula, which is known popularly as the allometry formula or equation,

$$y = bx^{\alpha},$$

where x and y represent respectively the body and the organ sizes; and b and α are two constants, known respectively as the 'initial growth index' and the 'equilibrium constant' or the 'growth-coefficient'.

There are, however, certain shortcomings in the allometry formula, which do not allow it to explain with great perfection many problems of relative growth. Thus the constant value of α, as given in this formula, does not properly explain the existence of growth-gradient within a limb. The growth-coefficient on the other hand should progressively change from point to point along the axis of an organ in order to fully justify the idea of a growth-gradient.

In the present paper an expression, depending on the size of the body, has been derived for the growth-coefficient.

Discussion

Huxley's assumption that,

$$\frac{1}{y}\frac{dy}{dt}\Big/\frac{1}{x} = \text{a constant } \alpha \qquad \dots \quad (1)$$

where x and y denote the sizes of the body and the organ respectively, t being the time factor, may be regarded as a first approximation to the more general assumption that,

$$\frac{1}{y}\frac{dy}{dt}\Big/\frac{1}{x}\frac{dx}{dt} = f(x) \qquad \dots \quad (2)$$

where $f(x)$ is a function of the body size. The form of $f(x)$ is to be derived now. This may be done as follows :

On integration with respect to x the equation (2) leads to,

$$y = c' \, e^{\int \frac{f(x)}{x} dx} \quad \dots \quad (3)$$

where c' is an arbitrary constant and e is the base of natural logarithms.

Similarly, integration of equation (1) leads to the well known equation,

$$y = bx^{\alpha},$$

or, on taking the logarithms,

$$\log_e y = \log_e b + \alpha \log_e x \quad \dots \quad (4)$$

Further, on arranging a sample data, giving the n values of the pair (x, y), arranged in order of magnitude of x, beginning with the smallest x, $(n-1)$ sets of values of the pair (x, y) are obtained on taking these two by two. Thus the first set will consist of the first and the second pairs, the second set of the second and the third pairs, and so on. On fitting the equation,

$$y = bx^{\alpha},$$

for each of these sets, $(n-1)$ values of the pair (b, α) are obtained. Hersh (1931, 1934) has shown that the relation holding between these values of b and α is of the form,

$$b = Be^{-r\alpha} \quad \dots \quad (5)$$

where B and r are constants.

Next, a curve may be fitted between the values of x and α. A sample of 90 males of the Indian freshwater prawns, *Palaemon hendersoni* DeMan was studied for this purpose. (The material was kindly lent to the author by the Director, Zoological Survey of India. It is deposited in the reserve collections of the Z. S. I.). It was seen that the relationship between α and x, within the limits of fluctuations due to sampling, is linear. Whether or not this straight line shows the best functional relationship between α and x can be decided only on a vast and extensive application of this analysis on different types of data; but in any case it may be taken to hold good at least as a first approximation, since a straight line is a type of relation which is always of importance and usefulness, being one of the simplest functions to fit and to explain.

The relationship between α and x is then expressible in the form,

$$\alpha = a_0 + a_1 x \quad \dots \quad (6)$$

where a_0 and a_1 are constants.

The nature of the function $f(x)$ can now be easily derived as follows :

Differentiation of equation (4) with respect to t leads, after slight rearrangement of terms, to

$$\frac{1}{y}\frac{dy}{dt}\Big/\frac{1}{x}\frac{dx}{dt} = \frac{x}{b}\frac{db}{dx} + \alpha + x \log_e x \frac{d\alpha}{dx} \quad \dots \quad (7)$$

Next, differentiation of equations (5) and (6) with respect to x gives

$$d\alpha/dx = a_1$$

and $$\frac{1}{b}\left[\frac{db}{dx}\right. = -r \, d\alpha/dx$$

$$= -ra_1$$

Substituting the values of $d\alpha/dx$ and $\frac{1}{b}\, db/dx$ in equation (7), it is seen that,

$$\frac{1}{y}\frac{dy}{dt}\Big/\frac{1}{x}\frac{dx}{dt} = -ra_1\, x+\alpha+ a_1\, x\log_e x$$

$$= a_1\, x(1-r)+a_0+a_1\, x\log_e x, \text{ from equation (6)}$$

Comparison of the right hand members of this equation and equation (2) gives,

$$f(x) = a_1\, x(1-r)+a_0+a_1\, x\, \log_e x$$

Hence,

$$f(x)/x = a_1(1-r)+a_0/x+a_1\, \log_e x$$

leading to,

$$e^{\int \frac{f(x)}{x} dx} = x^{(a_0+a_1x)}\, e^{-ra_1x}$$

Thus equation (3) may be written as,

$$y = c'\left[x^{(a_0+a_1x)}\, e^{-ra_1x}\right]$$

Fitting the regression equation between $\log_e y$ and $\log_e\left[x^{(a_0+a_1x)}\, e^{-ra_1x}\right]$

gives finally a relation which may be expressed as,

$$y = bx^{(\alpha+ax)}\, e^{cx} \qquad \dots \quad (8)$$

where b, α, a and c are constants.

(Equation (8) reduces to $y = bx^{\alpha}$, when $a=c=0$).

The growth relationship between the body size x and the organ size y is thus established.

The growth-coefficient is, therefore, given as,

$$\text{The growth-coefficient} = \frac{1}{y}\frac{dy}{dt}\Big/\frac{1}{x}\frac{dx}{dt}$$

which, from equation (8) $= \alpha+(a+c)x+ax\log_e x$

This may be defined as ρ.

This expression for ρ thus shows that the growth-coefficient is not independent of the body size for a range of values of x, however small. On the other hand it is a function of the body size.

Verification of the Law

The 90 values of the log. carapace length, X, and the log. length of the second cheliped (or its segments Ischium, Merus, Carpus, Propodus and Dactylus), Y, in the male prawns of *P. hendersoni* DeMan were condensed to a smaller number, 13, of classes, with equal intervals. Table I shows the values of group averages for X and Y.

Table I

(Showing the values of the group averages X and Y)

Group*	Average X	Average Y: Is.**	Mo.	Ca.	Pro.	Dac.	Ch.
		(First Phase)					
0.65—0.70	0.6902	0.3802	0.3010	0.2553	0.3424	0.3802	1.0334
0.70—0.75	0.7364	0.4150	0.3520	0.3323	0.3979	0.4232	1.0846
0.75—0.80	0.7764	0.4589	0.3867	0.3602	0.4370	0.4705	1.1236
0.80—0.85	0.8245	0.4928	0.4347	0.3918	0.4780	0.5120	1.1632
0.85—0.90	0.8745	0.5301	0.4773	0.4355	0.5260	0.5631	1.2078
0.90—0.95	0.9315	0.5729	0.5181	0.4830	0.5809	0.6244	1.2599
0.95—1.00	0.9745	0.6191	0 5807	0.5376	0.6533	0.6801	1.3145
1.00—1.05	1.0290	0.6685	0.6393	0.5792	0.7324	0.7671	1.3815
		(Second Phase)					
1.05—1.10	1.0763	0.7295	0.6944	0.6320	0.7995	0.8338	1.4431
1.10—1.15	1.1220	0.7750	0.7489	0.7012	0.8889	0.9132	1.5108
1.15—1.20	1.1781	0.8393	0.8342	0.7897	1.0205	1.0393	1.6166
1.20—1.25	1.2211	0.8926	0.9016	0.8496	1.1160	1.1166	1.6903
1.25—1.30	1.2565	0.9367	0.9859	0.9367	1.2133	1.2055	1.7737

* Groups have been formed according to X.

** Abbreviations : Is.—Ischium; Me.—Merus; Ca.—Carpus; Pro.—Propodus; Dac.—Dactylus and Ch.—Cheliped.

(It will be seen that there is a change of phase in the growth relationship at about the value 1.05 of X).

The following values of equation (8) have been derived from the contents of Table I :

(First Phase)

Ischium $y = 0.8987\, x^{(0.67854+0.02704x)}\, e^{-0.06006x}$

Merus $y = 0.7644\, x^{(0.78821+0.05575x)}\, e^{-0.14321x}$

Carpus $y = 0.2882\, x^{(1.05783-0.03945x)}\, e^{+0.09836x}$

Propodus $y = 2.3217\, x^{(0.40589+0.15517x)}\, e^{-0.37601x}$

Dactylus $y = 2.6564\, x^{(0.38025+0.15969x)}\, e^{-0.38597x}$

Cheliped $y = 4.7731\, x^{(0.73874+0.07066x)}\, e^{-0.17922x}$

(*Second Phase*)

Ischium $y = 2.2187\, x^{(0.31015+0.03874x)}\, e^{-0.08604x}$

Merus $y = 88.8581 x^{(-1.08553+0.15950x)}\, e^{-0.40973x}$

Carpus $y = 13.5650\, x^{(-0.44531+0.11665x)}\, e^{-0.29084x}$

Propodus $y = 3.0040\, x^{(0.27779+0.10663x)}\, e^{-0.25838x}$

Dactylus $y = 2.5010\, x^{(0.38496+0.08817x)}\, e^{-0.21310x}$

Cheliped $y = 0.2271\, x^{(1.92861-0.00620x)}\, e^{+0.01573x}$

Table II shows the observed (in columns a) and the calculated (in columns b values of the carapace length, x, and the lengths, y, of the cheliped and its five segments; rounded of to the first place of the decimals and expressed in millimeters.

TABLE II

(*Showing the observed, in columns 'a', and the calculated, in columns 'b', values of the group average lengths, for x and y, expressed in mm. and rounded of to the first place of decimals.*)

Average x	Average y											
	Is.		Me.		Ca.		Pro.		Dac.		Ch.	
	(First Phase)											
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
4.9	2.4	2.4	2.0	2.0	1.8	1.8	2.2	2.3	2.4	2.5	10.8	11.1
5.4	2.6	2.6	2.2	2.2	2.2	2.1	2.5	2.5	2.6	2.7	12.1	12.1
6.0	2.9	2.8	2.4	2.4	2.3	2.3	2.7	2.7	3.0	2.9	13.3	13.0
6.7	3.1	3.1	2.7	2.7	2.5	2.5	3.0	2.8	3.3	3.2	14.6	14.4
7.5	3.4	3.4	3.0	3.0	2.7	2.8	3.4	3.3	3.7	3.5	16.1	16.0
8.5	3.7	3.8	3.3	3.4	3.0	3.1	3.8	3.8	4.2	4.1	18.2	18.4
9.4	4.2	4.1	3.8	3.8	3.4	3.4	4.5	4.5	4.8	4.8	20.6	20.6
10.7	4.7	4.7	4.4	4.4	3.8	3.7	5.4	5.6	5.8	6.0	24.1	24.2
	(Second Phase)											
11.9	5.4	5.4	5.0	5.1	4.3	4.4	6.3	6.4	6.8	6.9	27.7	27.2
13.2	6.0	6.0	5.6	5.6	5.0	4.9	7.7	7.7	8.2	8.2	32.4	33.0
15.1	6.9	6.9	6.8	6.6	6.2	6.0	10.5	10.2	11.0	10.6	41.4	41.8
16.6	7.8	7.8	8.0	8.0	7.1	7.2	13.1	13.1	13.1	13.2	49.0	50.0
18.0	8.6	8.7	9.7	9.8	8.6	8.7	16.3	16.6	16.0	16.3	59.4	57.8

Note : In the above Table the sum of the lengths of the five segments for some groups may differ from the length of the corresponding cheliped because of rounding the resulting figures to the first place of decimals. Figures correct up to the second place of decimals are retained by the author.

SUMMARY

1. It has been established that the allometry equation, $y = bx^{\alpha}$, expressing the growth relationship between the body size x and the organ size y in an organic form, is only a first approximation of the following more general relationship between x and y :—

$$y = bx^{(\alpha+ax)}e^{cx},$$

where b, a, α and c are constants.

2. The existence of such a relationship between the carapace length x and the length y of the second pair of chelipeds (or their segments) has been verified from data on 90 male species of the Indian freshwater prawns, *Palaemon hendersoni* DeMan.

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ON THE PHYLLOSOMA OF MANDAPAM[1]

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Introduction

Several species of lobsters belonging to the families Palinuridae and Scyllaridae have been known to occur in Indian waters but the lack of information of their larvae is rather striking. This is perhaps because of the relative scarcity of these larvae in the plankton collections made in the inshore waters and probably also due to the difficulty in rearing them in captivity for a long time, for the duration of larval life is known to be long and may range from three to six or seven months. This long larval life is important in the life history of lobsters because this period covers the main dispersal phase of the species concerned and the distribution of the adult depends to a very large extent upon the duration of this larval life. Plankton investigators from various centres along the coasts of India have reported the occurrence of Phyllosoma in the inshore plankton and throughout they do not seem to be very common. No attempt has so far been made to classify the different types of Phyllosoma that occur in our waters and besides we know very little about the season of occurrence of these larvae at the various places. Alikunhi (1948) seems to have been able to study in detail the mechanism of larval and post-larval moults and the various stages in the development of two species of *Scyllarus* and a single species of *Panulirus*. According to him the Phyllosoma larvae form a conspicuous item, though not very common, of the macroplankton of the Madras coast particularly during March and that the early stages are remarkably few in the surface plankton, while the final pelagic stage predominates. He adds that the final pelagic larvae metamorphose into post-larvae overnight in the laboratory, the early larvae also moult into later stages and that the post-larval specimens thrive in aquaria and grow by regular moults. Unfortunately the results of his investigation are reported only in the form of a brief note and the details have not been published yet.

Three species of the family Palinuridae, *Panulirus ornatus* (Fabricius), *P. dasypus* (*Latreille*) and *P. fasciatus* (Fabricius) and one species of the family Scyllaridae *Thenus orientalis* (Rumph.) are commonly caught in this area. During 1950 to 1955 a number of Phyllosoma larvae, belonging to different types and stages of development, were obtained from the local plankton and it was thought that a study of these will be interesting and informative, particularly in view of our very meagre knowledge of these larvae. There is still a great deal of uncertainty about the identity of the different types of Phyllosomas and in many cases they have not been positively correlated with the adults. Similarly, in many species the number of stages through which the Phyllosomas pass before metamorphosing into the puerilla stage is also still mostly a matter of conjecture. Therefore, attempts were made to rear the larvae in the aquarium. It was possible to keep berried specimens of *P. ornatus* and *T. orientalis* in the aquarium until the eggs hatch and

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thus establish the identity of the first Phyllosoma, the characters of which are quite important in recognising the later stages. Efforts to keep the larvae in the aquarium to study the later larval history have, however, proved so far unsuccessful.

Descriptions of Phyllosoma

Panulirus ornatus (Fabricius)

A berried specimen was obtained on January 2, 1955. The eggs were almost spherical, 280μ in diameter and orange-red in colour at the time of capture. They gradually increased in size as development advanced and became 350μ when the embryos were practically fully developed. On February 16, the eggs hatched and the first Phyllosomas (Fig. 1A) were seen actively swimming about. The larvae had bright orange-red chromatophores distributed over the body and the appendages as shown in Fig. 1A. It has been reported by some of the earlier workers that there is a stage before the first Phyllosoma and that this stage lasts only for a few hours. Gilchrist (1913 and 1916 as quoted by Gurney, 1936) who recorded this for the first time in *Palinurus* (*Jasus*) *lalandii* gave the name "naupliosoma" to these larvae. He suggested that if the hatching takes place at night one may miss the naupliosoma and that this active stage is retained in order that the surface may be reached more rapidly than a normal Phyllosoma would be able to do. Von Bonde (1936) observed a pre-naupliosoma stage in this species which lasted for about eight hours before moulting into the naupliosoma. Sheard (1949) observed a naupliosoma stage in *Panulirus longipes*. He remarks that the naupliosoma is quickly followed by the first of a number of Phyllosoma stages and adds that the naupliosoma avoids light, creeping into rocks, cracks, etc., for protection. Gurney (1936) is of the opinion that the naupliosoma represents the prezoeal stage. Neither Lebour (1950) nor Lewis (1951), who made observations on *Panulirus argus*, have recorded these stages and according to them the eggs of *P. argus* hatch as first Phyllosomas. In the present instance also the authors have not noticed either a pre-naupliosoma or a naupliosoma stage. The first Phyllosoma stage was seen early in the morning and it is not unlikely that hatching took place at night and the naupliosoma stage escaped unnoticed. During the last few years several berried crabs, alpheids, etc., were kept under observation by the authors and it was their experience that hatching in most cases took place at night.

The first Phyllosoma of *P. ornatus* (Fig. 1A) measures 1.42 mm. in length (from the tip of the abdomen to the tip of the fore-body between the eyes) and 0.67 mm. across the widest part of the fore-body. The hind-body is slightly narrower than the fore-body. The eyes are thick stalked and are about as long as the first antennae or may be slightly shorter. First antenna is long and slender and is about one and a half times the length of the second antenna. There are three long terminal hairs and a short one. Towards the distal half there are a series of short hairs on either side. The second antenna is unsegmented with two short terminal hairs, two short ones on the outer side and a few on the inner side (Fig. 1B). Second maxilla is two jointed, the basal segment bears three short setae and the distal segment has four long plumose setae. The second maxillipede is five jointed and without an exopodite. The distal segment is prolonged into a spine and has two short hairs at the base of the spine. The penultimate segment bears four short setae (Fig. 1C). The third maxillipede has an exopodite. There are three pereiopods present of which only the first and second are biramous with well developed exopodite bearing natatory setae. The exopodite of the third pereiopod is visible as a rudiment. All the three pereiopods have prominent coxal spines (Fig. 1A). The abdomen is about half the length of the hind-body and the sides are almost

parallel. The tip of the abdomen is drawn out into points on either side, each side having three spines (Fig. 1D).

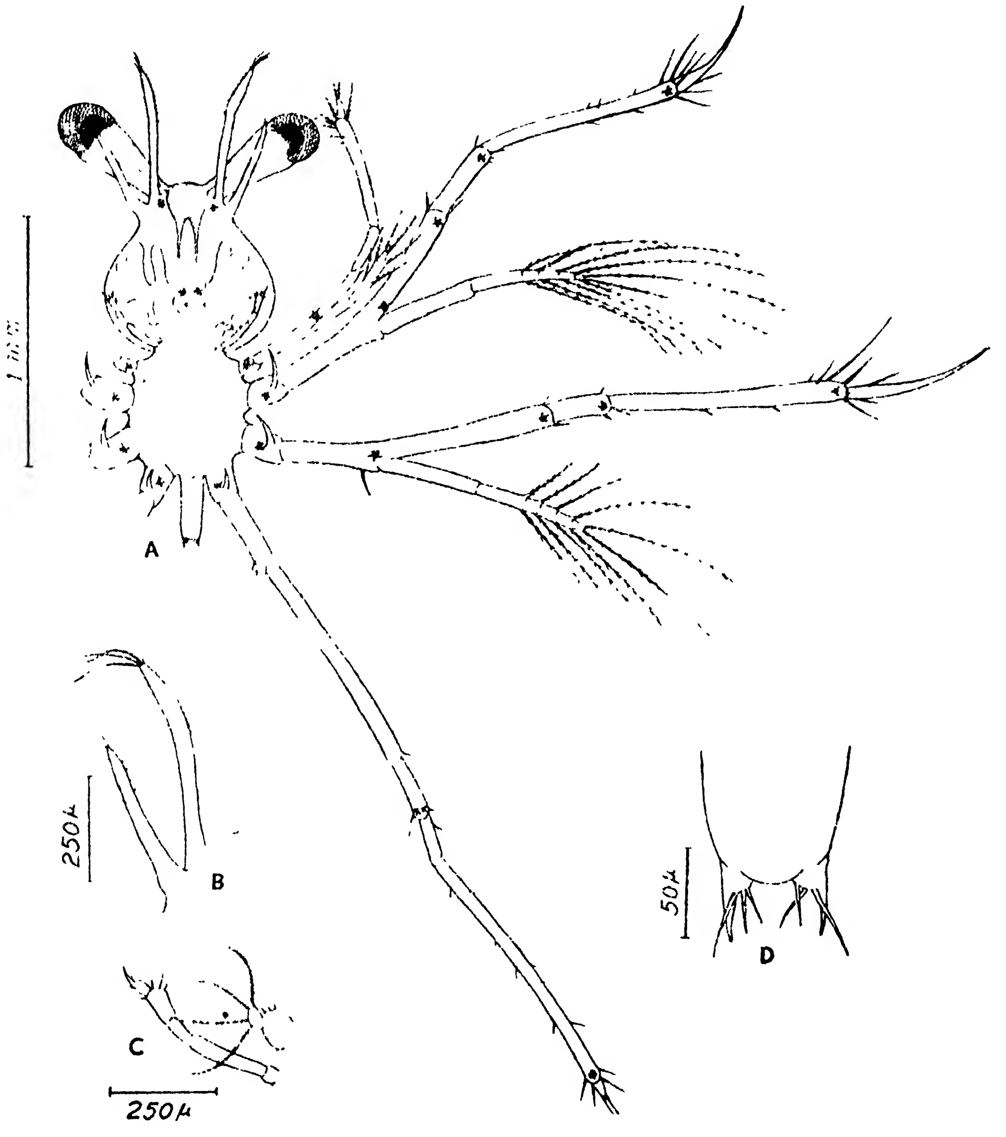


Figure 1. A. The first Phyllosoma of *Panulirus ornatus*. B. The first and second antennae. C. The second maxilla and the second maxillipede. D. The abdomen.

Gurney (1936) has given the characters of the Phyllosoma of *Panulirus* as follows :

"Fore-body pear-shaped, sometimes very narrow. Hind-body wider, sometimes much wider than fore-body; generally concave behind. Abdomen small and narrow in early stages."

"Antenna slender. Maxillipedes 2 and 3 with exopod in later stages. Leg 5 without exopod."

He separated the material he had into two groups one form (Form A) in which the hind-body is not much wider than the fore-body, antenna in early stage

much longer than antennule, exopodite of the maxilla enlarging early and always with setae and the third maxillipede and the first pereiopod having coxal spines and Form B with hind-body much wider. antenna in early stage shorter than the peduncle of the antennule, exopodite enlarging late and without setae until last stage and no coxal spines on the third maxillipede and first pereiopod.

Gurney (1936) has also described a third type (Form D) which he thinks may be of *Panulirus*. These have the fore-body pear-shaped, narrow in front, narrower than hind-body and which is slightly hollowed behind in later stages. The antennule has segments two and three of peduncle nearly equal and about half the length of the first segment. Antenna is slender and longer than the antennule in the earliest stages. The maxillule is without palp and the exopodite of the maxilla is setose. The second maxillipede is without an exopodite in the early stages but has a setose exopodite later, whereas the third maxillipede has a setose exopodite from the earliest stage. Pereiopods have large ventral coxal spines and the second and third pereiopods have large dorsal coxal spines. The dactylus of the second pereiopod is elongated.

The characters of the Phyllosoma under discussion do not agree completely with those of either of the forms described by Gurney (1936). The present larvae differ from Form A in that the hind-body is slightly narrower than the fore-body, antennae are shorter than the antennules and the third maxillipede is without a coxal spine, while they differ from Form B in not having the hind-body much wider than the fore-body and the presence of coxal spines in the first pereiopod. There are also differences from Form D of Gurney, the identity of which he himself is not certain. It should, however, be pointed out that Gurney (1936) had no corresponding stage in his collection and unfortunately the present authors do not have later stages in their collection. The present Phyllosoma, it may be added, shows many characters which Gurney (1936) has described for the Phyllosoma of *Palinurus*. In fact it agrees more with the characters of *Palinurus* rather than *Panulirus* as given by Gurney. They are (1) fore-body about as wide as long at all stages and wider than hind-body, (2) hind-body not concave behind, (3) third maxillipede with setose exopodite from the first stage, (4) abdomen parallel sided, (5) the dactylus of the first and second pereiopods prolonged into very long spines, and (6) pereiopods with coxal spines. Here again Gurney had access only to comparatively late stage of larvae. The first Phyllosoma of *P. ornatus*, however, shows close resemblance to the corresponding stage *P. argus* (see Lebour, 1950 and Lewis, 1951) and to that of *P. interruptus* and *P. gracilis* (see Johnson, 1956). There is, however, no uncertainty about the identity of the Phyllosoma described above because these larvae hatched in the aquarium as mentioned earlier.

Thenus orientalis (Rumph.).

A berried specimen of *T. orientalis* was obtained on July 8, 1953 and eggs were in a fairly advanced stage of development. The eggs were dull yellowish-brown in colour and measure 830μ in diameter. The larvae hatched on July 20.

Phyllosoma I (Fig. 2A).

The larvae measure 2.95 mm. from the front margin of the carapace to the tip of the abdomen. The fore-body which is broader than long is much broader than the hind-body. The latter is almost circular and is 1.29 mm. wide. The former is 2.19 mm. at its widest part. The eyes have a fairly thick stalk and are slightly longer than the first antenna (Fig. 2A). The first antenna is long, slender and unsegmented with three terminal hairs. A little above the middle of the antenna there is a short spine. The second antenna is less than half the length of the first. It is unsegmented and bears two terminal setae (Fig. 2B). The second maxilla is single jointed with three plumose setae. The second maxillipede is without

an exopodite and consists of five segments of which the second is the longest. The terminal segment tapers to a point and has two short setae. The third and fourth segments carry one and five setae respectively (Fig. 2C). The third maxillipede is uniramous. Pereiopods one, two and three are biramous with the exopodite having well developed natatory setae. The dactylus of the first, second and third pereiopods are not very elongated. The fourth pereiopod is uniramous. Coxal spine is present on the maxillipede and the first four pereiopods. The fifth pereiopod is present as a rudiment. It is unsegmented and is about two-thirds the length of the abdomen (Fig. 2A). The abdomen is rather small and is about half the length of the hind-body. The corners of the abdomen are drawn out into points on either side with three bristles on either side (Fig. 2D).

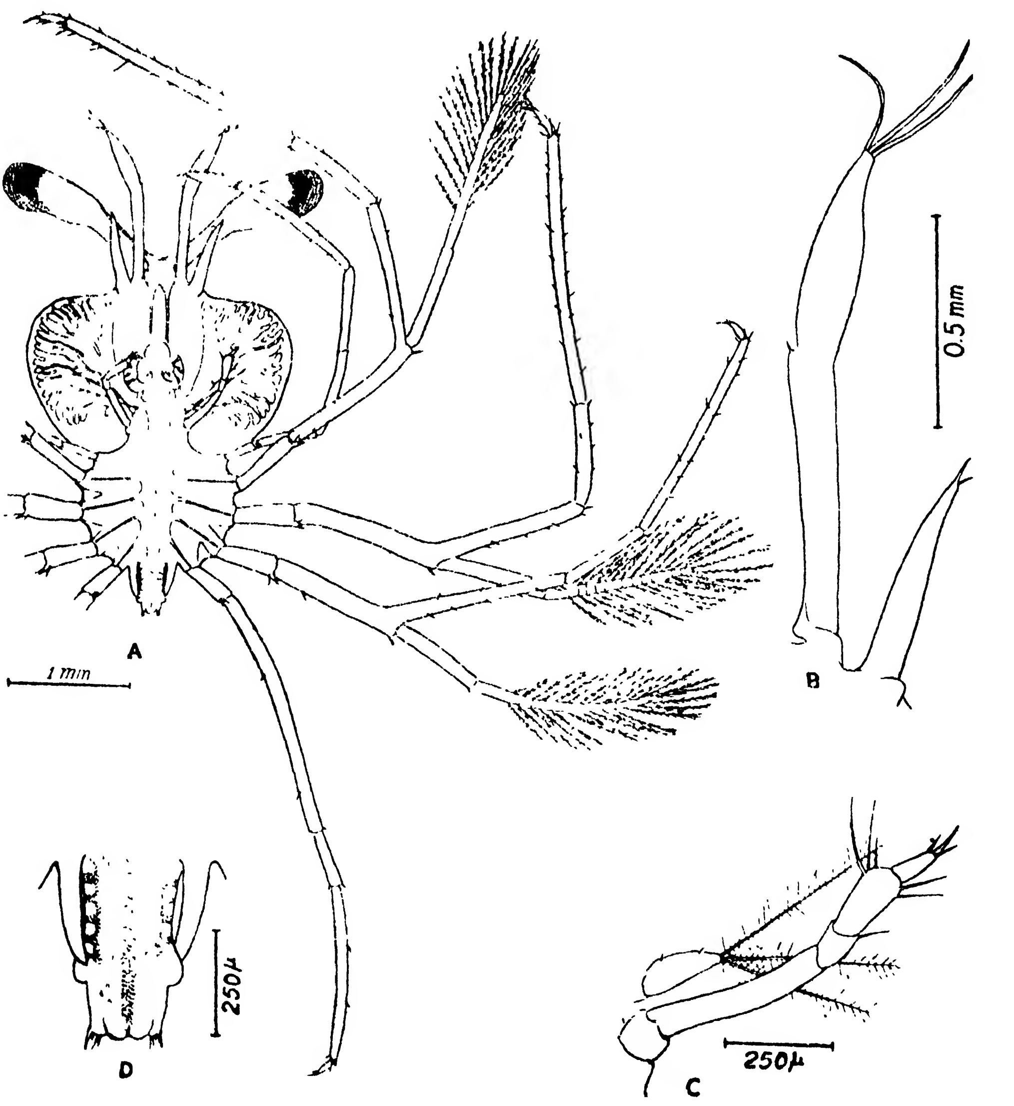


Figure 2. A. The first Phyllosoma of *Thenus orientalis*. B. The first and second antennae. C. The second maxilla and second maxillipede. D. The abdomen and the rudimentary fifth leg.

*Phyllosoma II**

This and the subsequent stages of Phyllosoma have been obtained from the plankton of the Mandapam area.

The Phyllosoma (Fig. 3) now measure 3.29 mm. in length. This stage does not show many striking differences from the first stage. The fore-body is little more than one and a half times the width of the hind-body. The eyes have become slightly longer and the eye-stalk more slender. The first antenna shows indications of segmentation at the place where the short spine is present. The exopodite is present in the fourth pereiopod as an unsegmented rudiment. The fifth pereiopod is still short and unsegmented and is about two-thirds the length of the abdomen. The base of the abdomen has become slightly broader.

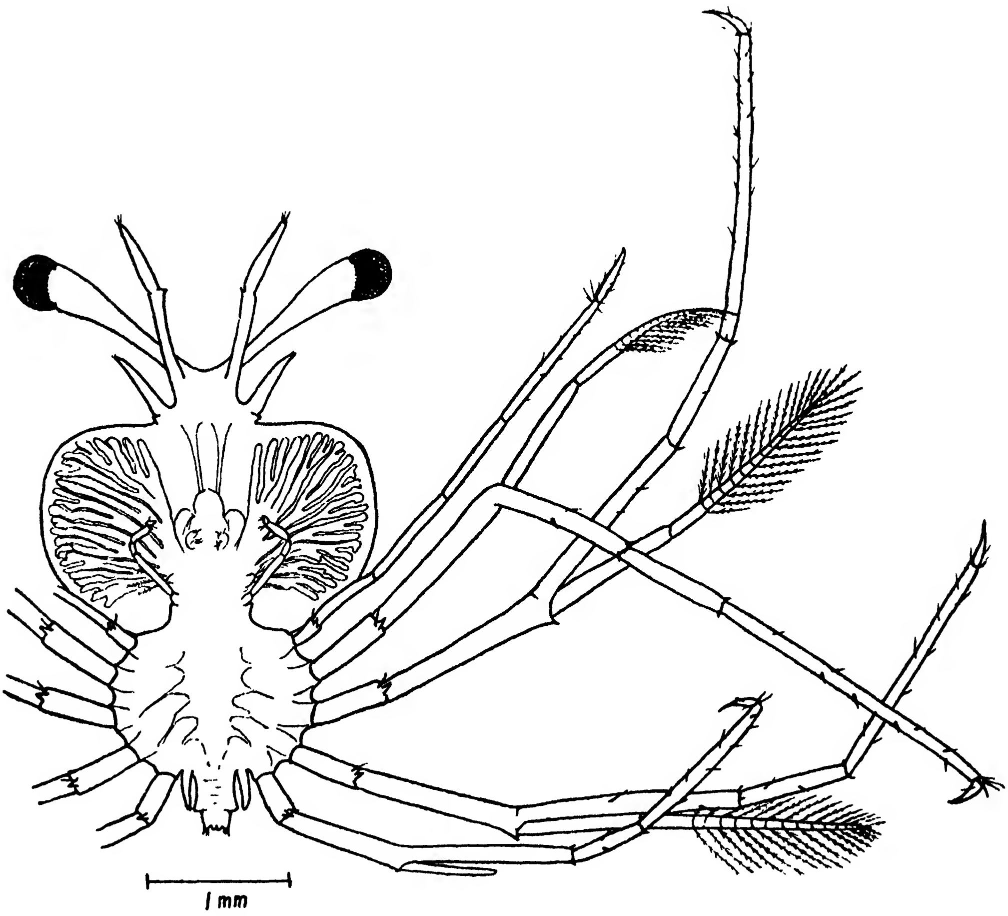


Figure 3. Phyllosoma II of *Thenus orientalis*.

Phyllosoma III (Fig. 4A)

The larvae measure 5.8 mm. in length. The fore-body has become very broad, almost twice as broad as the hind-body. The eye-stalk now shows two distinct segments, the long, cylindrical, proximal part connected by articulation

*The numbers do not refer to the actual stages in the development as the number of Phyllosoma stages in the development of the species is not known.

with the distal, conical part. It is now much longer than the first antenna. In the first antenna the short spine has become greatly enlarged and appears like a diverticulum. It is still unsegmented. In addition to the terminal setae it has developed four rows of sensory setae. The second antenna now appears bifurcate, somewhat longer being nearly two-thirds in length of the first antenna (Fig. 4B). The second maxilla has lost its plumose setae and also slightly changed in its shape. The first maxillipede has appeared as a small protuberance between the second maxilla and the second maxillipede. The second maxillipede (Fig. 4C) remains essentially the same as in the previous stage. There is no change in the third maxillipede as well as in the first, second and third pereiopods. The exopodite of the fourth pereiopod has become jointed and possesses natatory setae. The fifth pereiopod is present and is nearly double the length of the abdomen. The abdomen has increased in width, the rudimentary uropods are distinctly indicated and are bifid. The telson has two short spines at the corners (Fig. 4D).

The nature of developmental changes that have taken place in Phylosoma III compared to the previous stage described here, suggests that what is described here as Phyllosoma III may actually represent the fourth stage in development and that stage III is not represented in the collection.

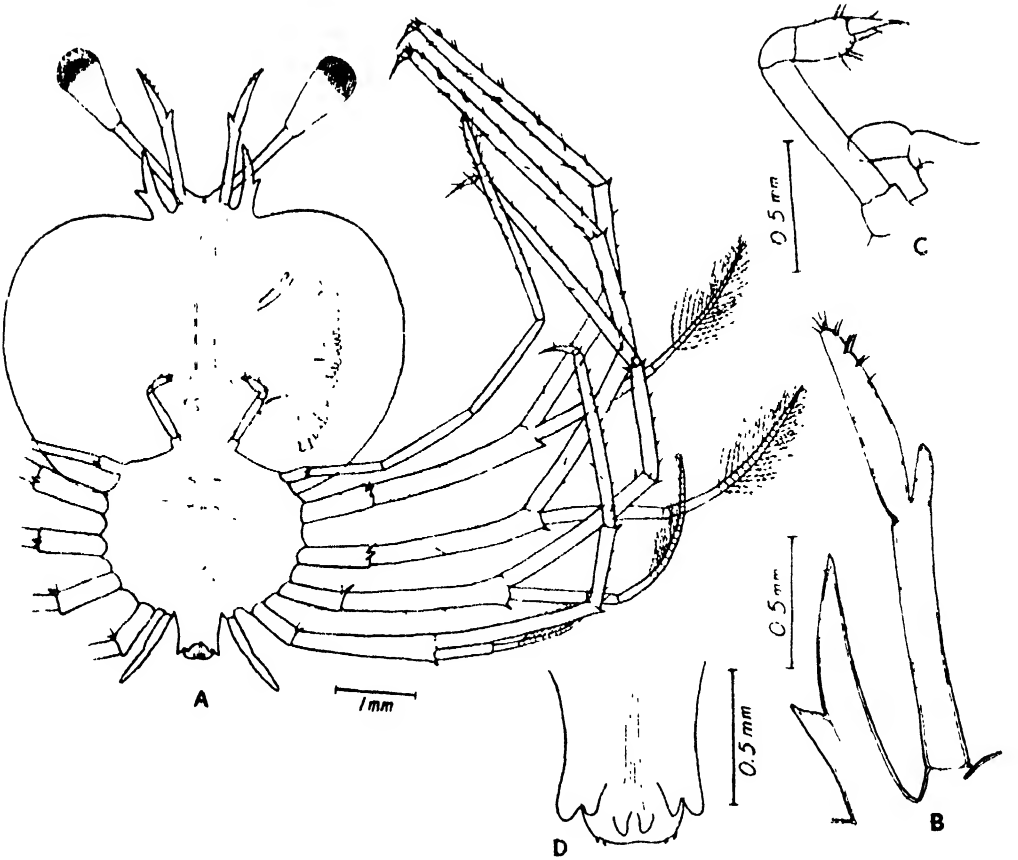


Figure 4. A. Phyllosoma III of *T. orientalis*. (Drawn from a whole mount). B. First and second antennae. C. The maxilla, the rudiment of the first maxillipede and the second maxillipede. D. Abdomen showing the rudimentary bifid uropods.

Phyllosoma IV

The next stage available is relatively very much advanced where the larvae measure 17.8 mm. in length (Fig. 5A). The fore-body has become very broad (13.6 mm.) while the hind-body measures only 7.2 mm. The first antenna is five jointed with a three jointed peduncle. The inner process at the end of the peduncle (the inner flagellum) is distinctly articulated at its base and it possesses a few marginal hairs. The outer process which becomes the outer flagellum has developed many more sensory setae. Both these segments are nearly of equal length. The second antenna has become broad and leaf-like with a strong process on the outer side and is almost as long as the first antenna. It shows signs of segmentation and the margin at the distal part as well as the margin of the lateral process are serrated (Fig. 5B). The second maxilla has the exopodite expanded but without setae. The first maxillipede is still a finger-like protuberance but with the indication of an epidopite (Fig. 5C). The second and third maxillipedes are with rudimentary exopodites without setae (Figs. 5A and C). The fifth pereiopod is longer than the abdomen and has six distinct segments. Gills are present on the third maxillipede and the first four pereiopods. The abdomen has become very much longer and there are indications of segmentation. These are especially distinct in the middle region of the abdomen but does not extend out to the sides. The uropods almost resemble those of the adults. All the pleopods are present as small protuberances. The telson shows two short lateral spines (Fig. 5D).

Subsequent stages are not available but it is likely that this may represent a fairly advanced stage and that there may be only two or three more stages before the Phyllosoma metamorphoses into the puerilla stage.

Stephensen (1923) has described the Phyllosoma of what he believes to be of *T. orientalis* from a single specimen. According to his description the carapace of the head is pear-shaped, somewhat longer than broad and the thorax nearly as long as broad with a notch in the posterior margin in which the short abdomen is set. The oral parts, according to him, resemble those of *Scyllarus arctus* at the same stage. Gurney (1936) has described six stages, stages IV to IX of Phyllosoma of *Thenus* and according to him the general characters are "Fore-body pear-shaped narrow in front in early stages, about 1½ times as wide as long; wider than hind-body. Hind-body deeply concave behind. Abdomen small and narrow."

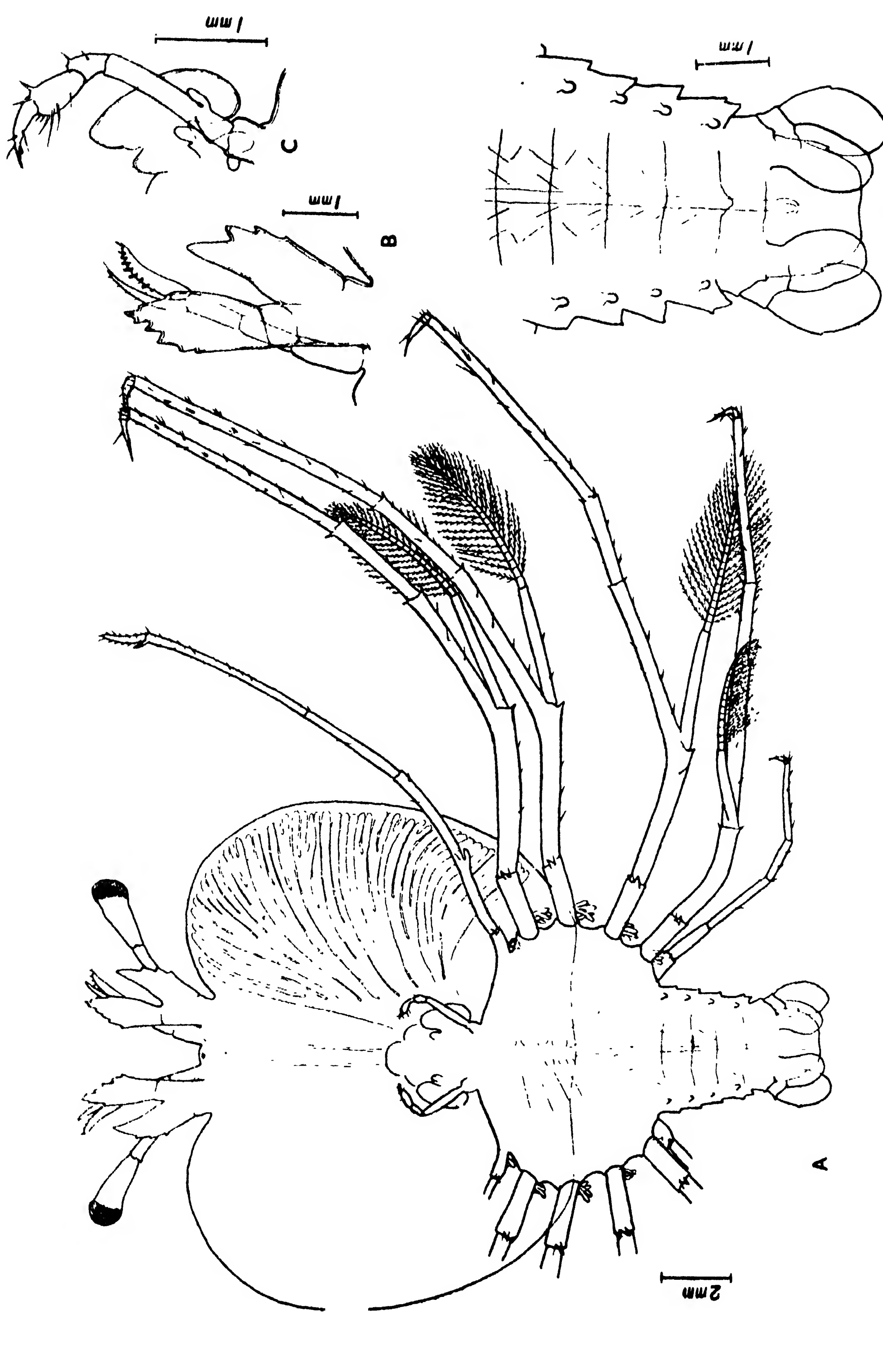
"Antennule with segments 2 and 3 equal, not much shorter than segment 1. Antenna short and stout, with strong pointed process on outer side of segment 2 of peduncle, segment 3 very much narrower than segment 2 in late stages."

"Maxillule without palp. Maxilla without setae on exopod. Maxillipedes 2 and 3 without functional exopods. Legs without coxal spines. Leg 3 with propodus dilated at end. Leg 5 without exopod, but with small rudiment of it in late stages."

It will be seen that the characters of the larvae of *T. orientalis* described here differ from those described by Stephensen (1923), the most significant difference being the shape of the fore- and hind-body and that of the abdomen. Similarly, the striking differences from the Phyllosoma of *Thenus* described by Gurney (1936) are the shape of the fore- and hind-body and the presence of coxal spines. However, the identity of the present series, at least that of the first Phyllosoma, is definitely known as the larvae hatched from a berried specimen kept in the aquarium and as the other three stages of Phyllosoma described here agree in all characters with the first Phyllosoma of *T. orientalis*, they seem to belong unquestionably to the same species.

Scyllarus. sp.

A third type of Phyllosoma was obtained from the plankton of the Mandapam area and in the entire collection there were seven distinct stages of development.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C
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B
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D
A
2 mm

From the general characters these undoubtedly appear to belong to the genus *Scyllarus*. The general characters of the Phyllosoma of the genus *Scyllarus* have been given by Gurney (1936) as founded on the description of *S. arctus*, based on the accounts given by Dohrn (1870 as quoted by Gurney) and Hornell (1894 as quoted by Gurney) both of whom described the first stage of *S. arctus* hatched from eggs and also by Stephensen (1923) who described the complete series of nine stages. The fore-body is much wider than long and much wider than the hind-body. Hind-body is not concave behind. Abdomen, in later stages, is very broad at the base and forms a direct continuation of the hind-body. Antenna is at first very much shorter than the antennule, later becoming broad at the base and with large outer pointed process; flagellum short and broad. Maxillule without palp. Exopodite of the maxilla with setae in the first stage but without setae in late stages. Second maxillipede without exopodite, or with exopodite rudimentary. Third maxillipede without exopodite. The dactylus of the second pereiopod not greatly elongated. The fifth pereiopod is without an exopodite and the pereiopods are with coxal spines. Pleopods in the last stage are very narrow and without appendix interna. The telson is with a large spine on either side.

Phyllosoma I (Fig. 6A)

The larvae measure 1.35 mm. in length. Stephensen (1923) has mentioned that the first Phyllosoma of *S. arctus* measures 1.5 mm. The fore-body is wider than long and much wider than the hind-body. The fore-body measures 0.54 mm. The eyes are thick stalked. The first antenna is longer than the eyes and at the commencement of the extreme third of its length there is a thin, long process. There are four terminal hairs one of which is much shorter than the other three. The second antenna is only about one-third the length of the first antenna. Both the first and the second antennae are unsegmented (Fig. 6B). The second maxilla consists of two joints, a long basal one and a small apical one which bears four plumose setae. The second maxillipede consists of five segments (Fig. 6C) and in general it resembles the second maxillipede of *Panulirus* and *Thenus*. The third maxillipede is without an exopodite. The first, second and third pereiopods are present of which the first two are biramous with a well developed exopodite bearing natatory setae. The third pereiopod has no exopodite but the second joint has at its distal end a short, thick process which may be the rudimentary exopodite. In the first Phyllosoma of *S. arctus* Stephensen (1923) has recorded a similar process. The rudiments of the fourth pair of pereiopods are seen as small rounded protuberances. All the pereiopods have coxal spines. The abdomen is short, slightly broader at the base and the corners are drawn out into a fairly long point on either side each with three setae (Fig. 6D).

EXPLANATION OF THE FIGURE 5.

Figure 5. A. Phyllosoma IV of *T. orientalis* (Drawn from a whole mount). B. The first and second antennae. C. The second maxilla and the second and third maxillipedes. D. The abdomen.

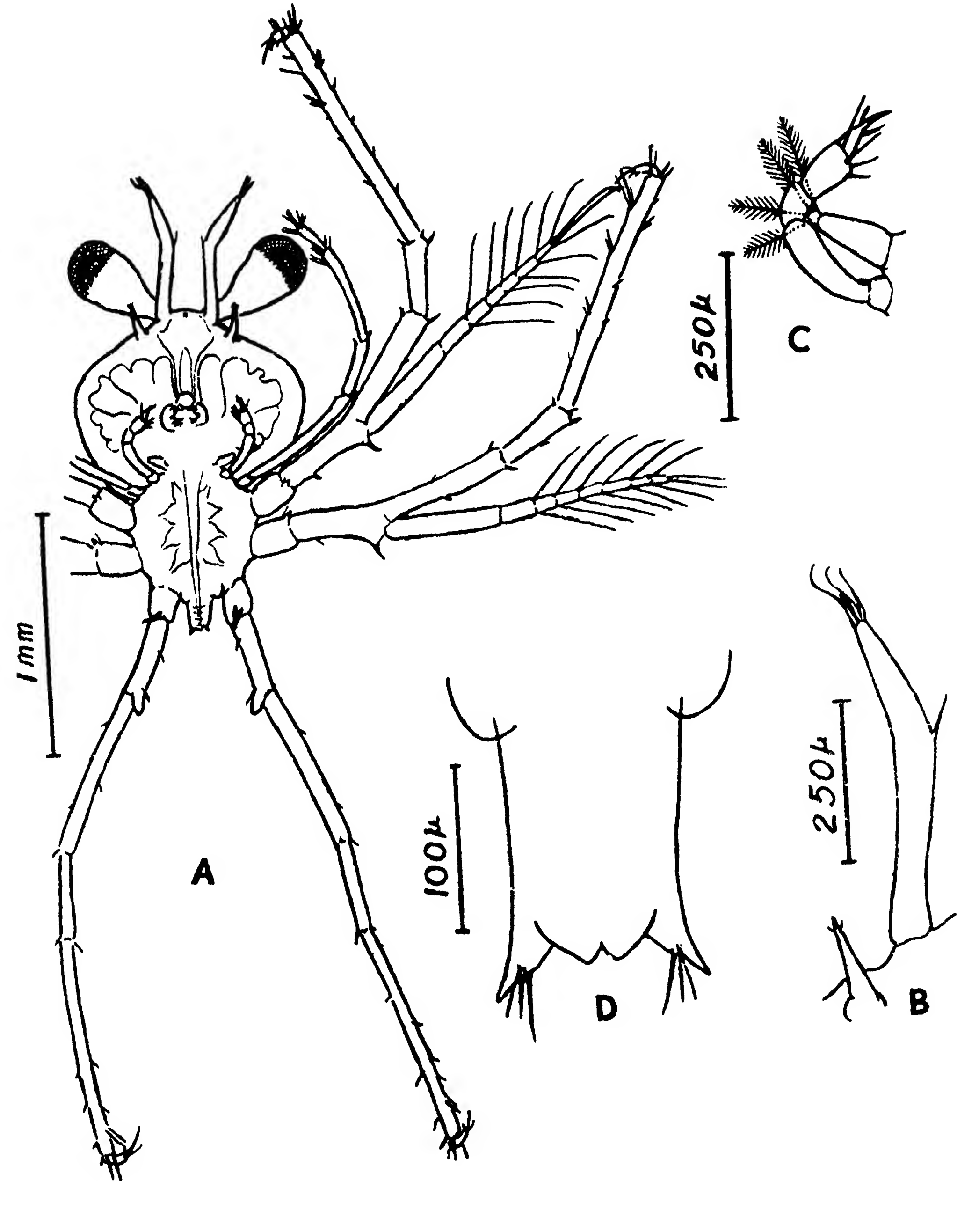


Figure 6. A. Phyllosoma I of *Scyllarus* sp. B. The first and second antennae. C. The second maxilla and the second maxillipede. D. The abdomen.

Phyllosoma II (Fig. 7A)

The larvae measure 1.83 mm. in length. There is a slight change in the shape of the fore-body which has now become more or less as long as it is broad. The eye-stalk has become thinner with a proximal cylindrical part and a distal somewhat conical part and the eyes are longer than the first antennae. Towards the tip of the first antenna a row of sensory setae has developed and except for this it resembles that of the previous stage (Fig. 7B). The second maxilla and the second maxillipede remain essentially the same as in the previous stage (Fig. 7C). The third pereiopod has an unsegmented exopodite. The rudiments of the fourth pair of pereiopods have appeared and they are longer than the abdomen. The tip of the abdomen shows very little change from that of the earlier stage (Fig. 7D).

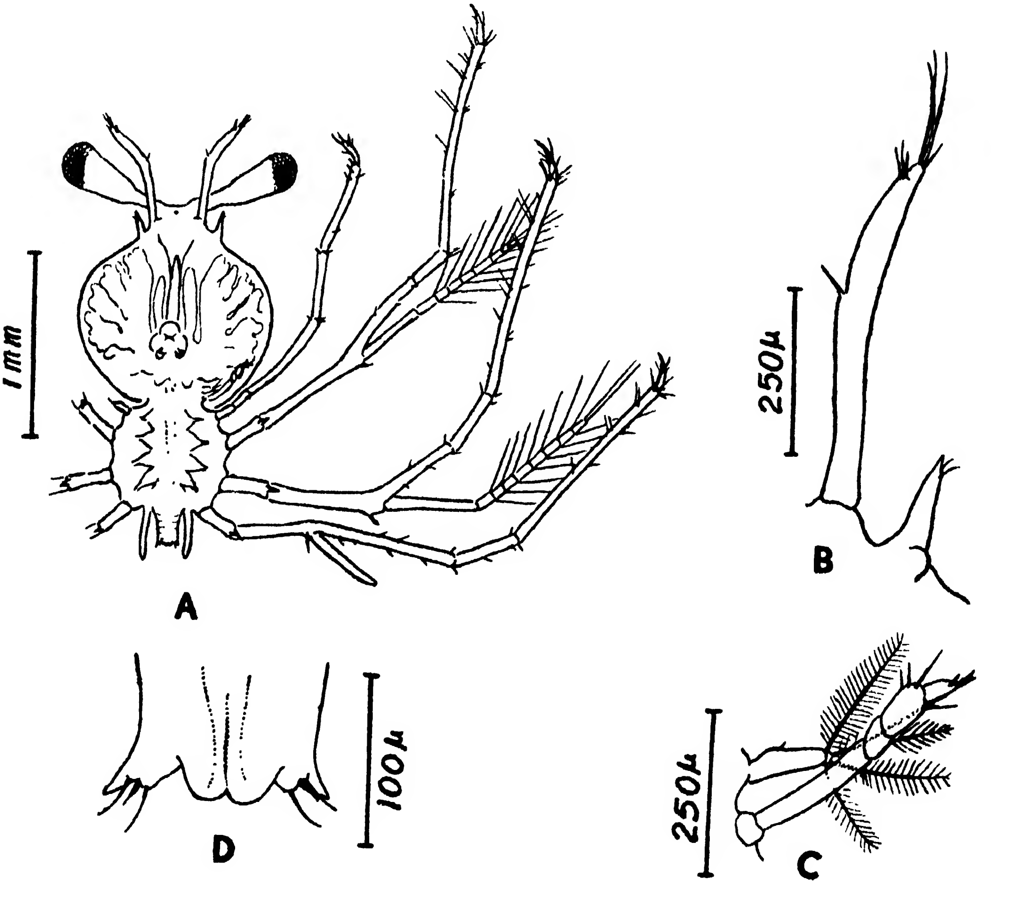


Figure 7. A. Phyllosoma II of *Scyllarus* sp. B. The first and second antennae. C. The second maxilla and the second maxillipede. D. The abdomen.

Phyllosoma III (Fig. 8A)

The larvae measure 2.65 mm. in length. The fore-body is a little longer than broad. The eyes are longer than the first antennae and the eye-stalk now consists of a distinct narrow, proximal, cylindrical part articulated with the distal, conical part. More sensory setae have developed on the first antenna. The process which was present at the commencement of the extreme third of the first antenna has now greatly thickened. The second antenna has slightly increased in length (Fig. 8B). The second maxilla and second maxillipede remain essentially the same as in the previous stage (Fig. 8C). The exopodite of the third pereiopod is fully developed with natatory setae. The fourth pereiopod has grown longer and the exopodite is present as a small protuberance. The tip of the abdomen shows no change from that of Phyllosoma II.

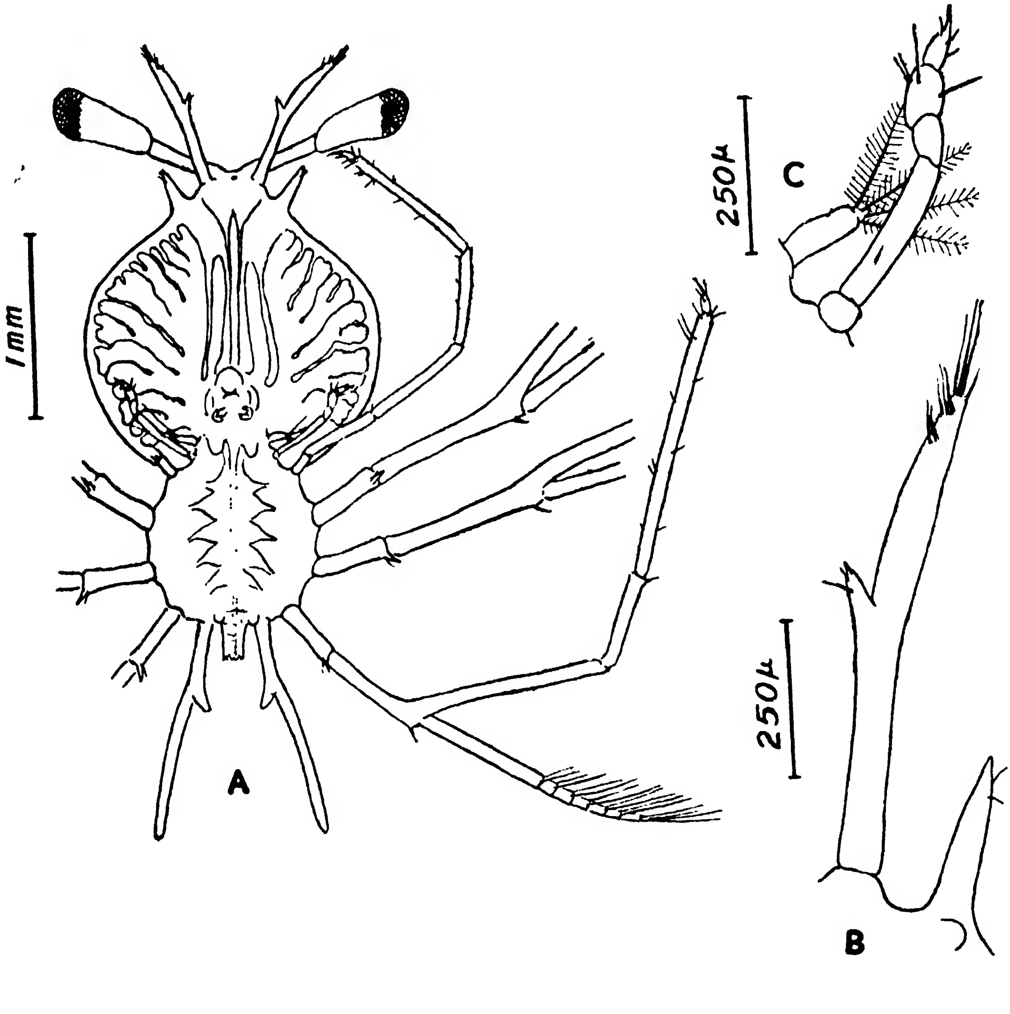


Figure 8. A. Phyllosoma III of *Scyllarus* sp. B. The first and second antennae. C. The second maxilla and the second maxillipede.

Phyllosoma IV

The next stage available in the series is the larvae measuring 2.97 mm. (Fig. 9A). In general appearance they resemble very closely the Phyllosoma of the previous stage described. The fourth pereiopod has increased in length and segmentations are clearly visible. The most important change is in the abdomen. The base of the abdomen has become much broader and the rudiments of the uropods are distinctly seen as a pair of protuberances (Fig. 9B).

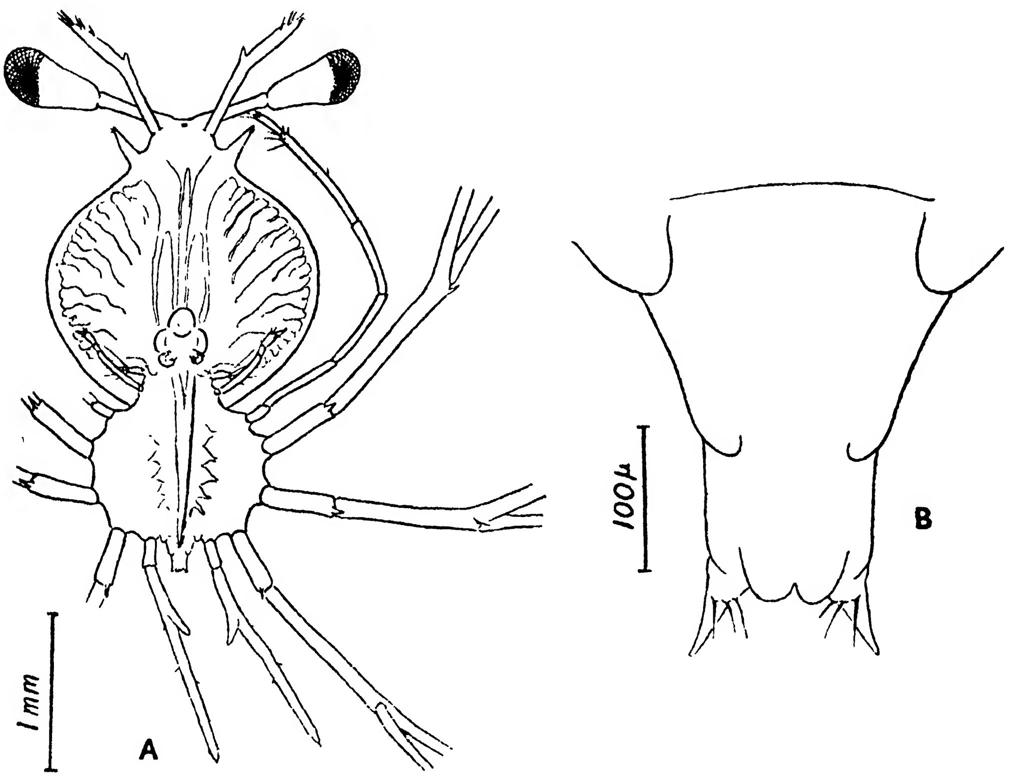


Figure 9. A. Phyllosoma IV of *Scyllarus* sp. B. The abdomen.

Phyllosoma V (Fig. 10A)

The larvae measure 3.31 mm. in length. The eyes are a great deal longer. The first antenna shows more sensory setae and also indications of segmentation. The inner process has now grown bigger. The second antenna is longer and is very nearly half the length of the first antenna (Fig. 10B). The second maxilla and the second maxillipede remain unchanged (Fig. 10C). The fourth pereiopod has increased very much in length, the segmentations are clearly indicated and the exopodite has become longer but without natatory setae. The rudiments of the fifth pereiopods are clearly indicated. The abdomen does not show any marked differences from that of Phyllosoma IV except that the rudiments of the uropods have become more prominent (Fig. 10D).

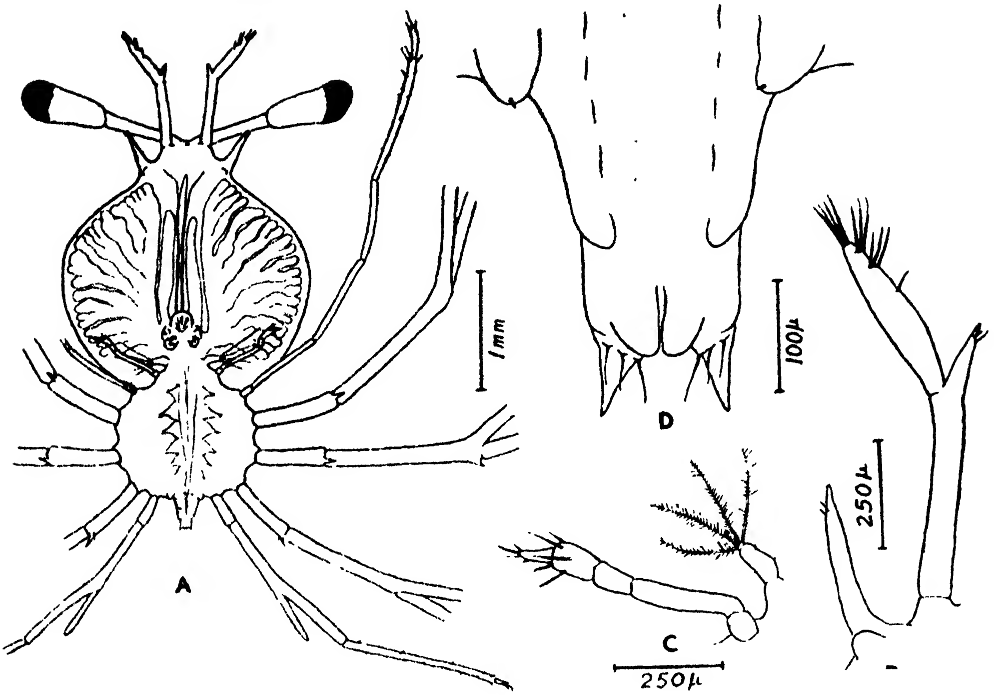


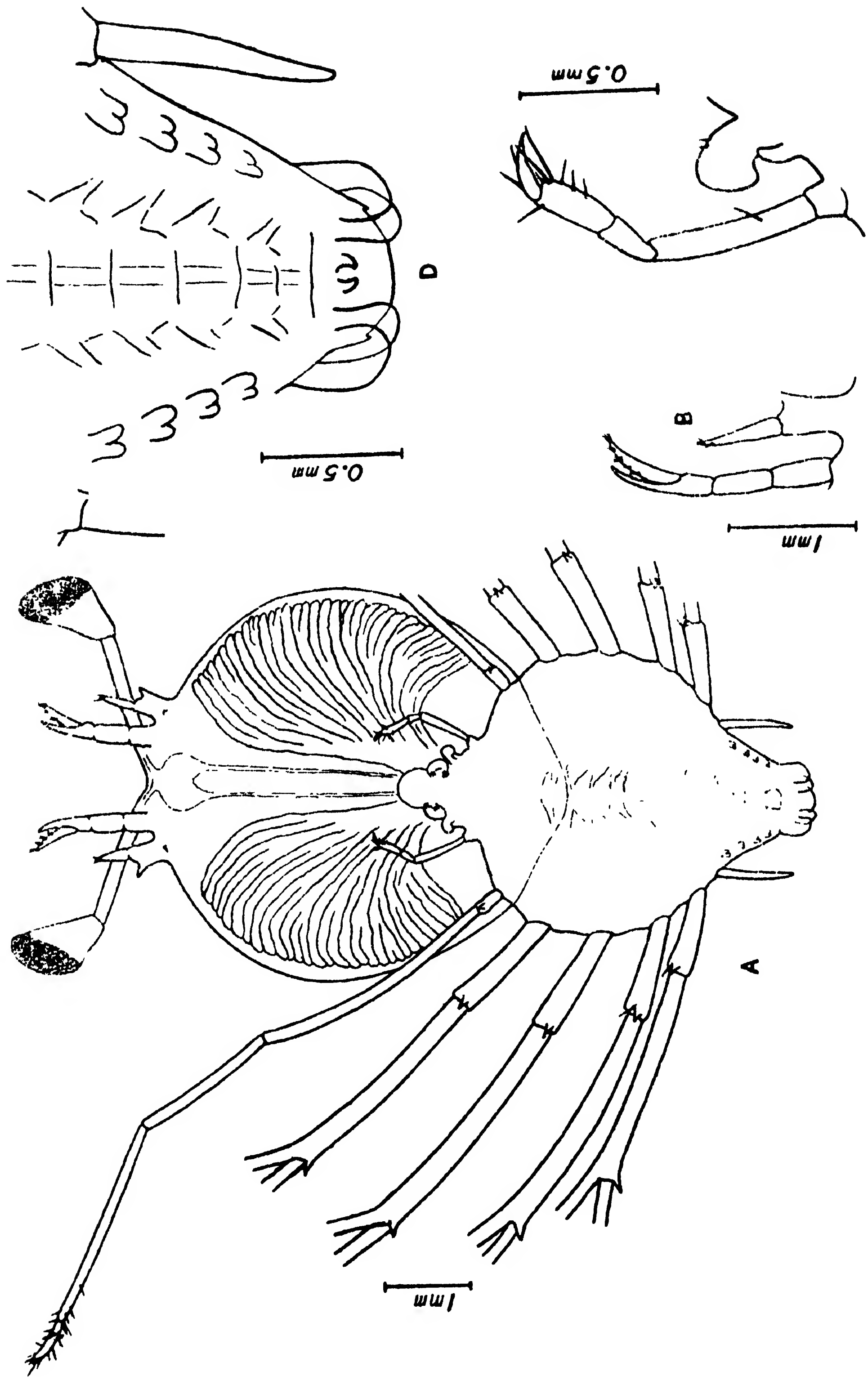
Figure 10. A. Phyllosoma V of *Scyllarus* sp. B. The first and second antennae C. The second maxilla and the second maxillipede. D. The abdomen.

Phyllosoma VI

The Phyllosoma available in the next stage of development measures 8.02 mm. (Fig. 11A). The eyes have become very much longer. The peduncle of the first antenna is three jointed and the first antenna has many more sensory setae. The second antenna has become longer and broader and is distinctly two segmented. The distal segment is somewhat conical and the basal segment has developed a process on the outer side (Fig. 11B). The second maxilla has changed markedly. It has become broad and leaf-like and has lost its small apical joint and the plumose setae. The first maxillipede has now appeared and is in the form of a finger-like process. The second maxillipede remains essentially similar to that of the earlier stages (Fig. 11C). The exopodite of the fourth pereiopod is fully developed and has the natatory setae. The fifth pereiopod is rudimentary but is almost as long as the abdomen. The base of the abdomen has become very much broader and forms a direct continuation of the hind-body. The uropods have increased in length and are deeply cleft. The telson is clearly marked off now and it has two short spines on either side. The pleopods are indicated as bifid processes. The abdomen shows faint indications of segmentation in the middle (Fig. 11D).

Explanation of the figure 11.

Figure 11. A. Phyllosoma VI of *Scyllarus* sp. B. The first and second antennae. C. The second maxilla, the rudiment of the first maxillipede and the second maxillipede. D. The abdomen.



D
0.5 mm
0.5 mm
C
B
1 mm
A
1 mm

Phyllosoma VII

The last stage in the series of Phyllosomas belonging to this type measures 14.30 mm. The fore-body has slightly changed its shape. The eyes have become still longer (Fig. 12A). The first antenna has a peduncle of three segments. The process which is to become the inner flagellum has developed a few hairs. It is still not articulated at its base. The second antenna has become broader at the base and is longer. The distal segment, which was conical, has become flat and leaf-like, the upper half of the inner margin having serrations. The lateral process on the second antenna has also increased in size (Fig. 12B). The second maxilla has a large exopodite. The first maxillipede remains as a finger-like process but with an epipodite. The second maxillipede has a rudimentary exopodite (Fig. 12C). The fifth pereiopod is five jointed and is almost as long as the abdomen. Stephensen (1923) has noticed in *S. arctus* that in most specimens corresponding to this stage the fifth pereiopod has only four joints but in some specimens there are five, the dactylus forming a segment by itself. The abdomen has grown considerably. There are clear indications of segmentation particularly in the middle region of the abdomen. The uropods have become larger and the exopodite is distinctly articulated. The two short spines on the telson are still present. The pleopods are much longer and deeply cleft (Fig. 12D).

The characters of the seven stages of Phyllosoma described above show close agreement with the larvae of *S. arctus* described by Stephensen (1923). According to him there are nine Phyllosoma stages in all but in some, however, the difference between the previous and succeeding stages is only slight. The stage of development of the fourth and fifth pereiopods and the abdomen with corresponding pleopods and uropods seems to give the best means of distinguishing the stages because the eyes, antennae and oral parts do not seem to afford quite such good characters for determining the age, as these do not altogether keep pace in regard to development with the pereiopods and the abdomen. On this basis the present collection consists of seven distinct stages. Phyllosomas I to V correspond to stages I to V of *S. arctus* described by Stephensen, whereas Phyllosoma VI and VII in the series seem to correspond to stages VII and VIII of Stephensen. Phyllosma VIII is fairly advanced and it is likely that in this species of *Scyllarus* also there are only nine stages in all, as in *S. arctus* in which case stages VI and IX alone are missing.

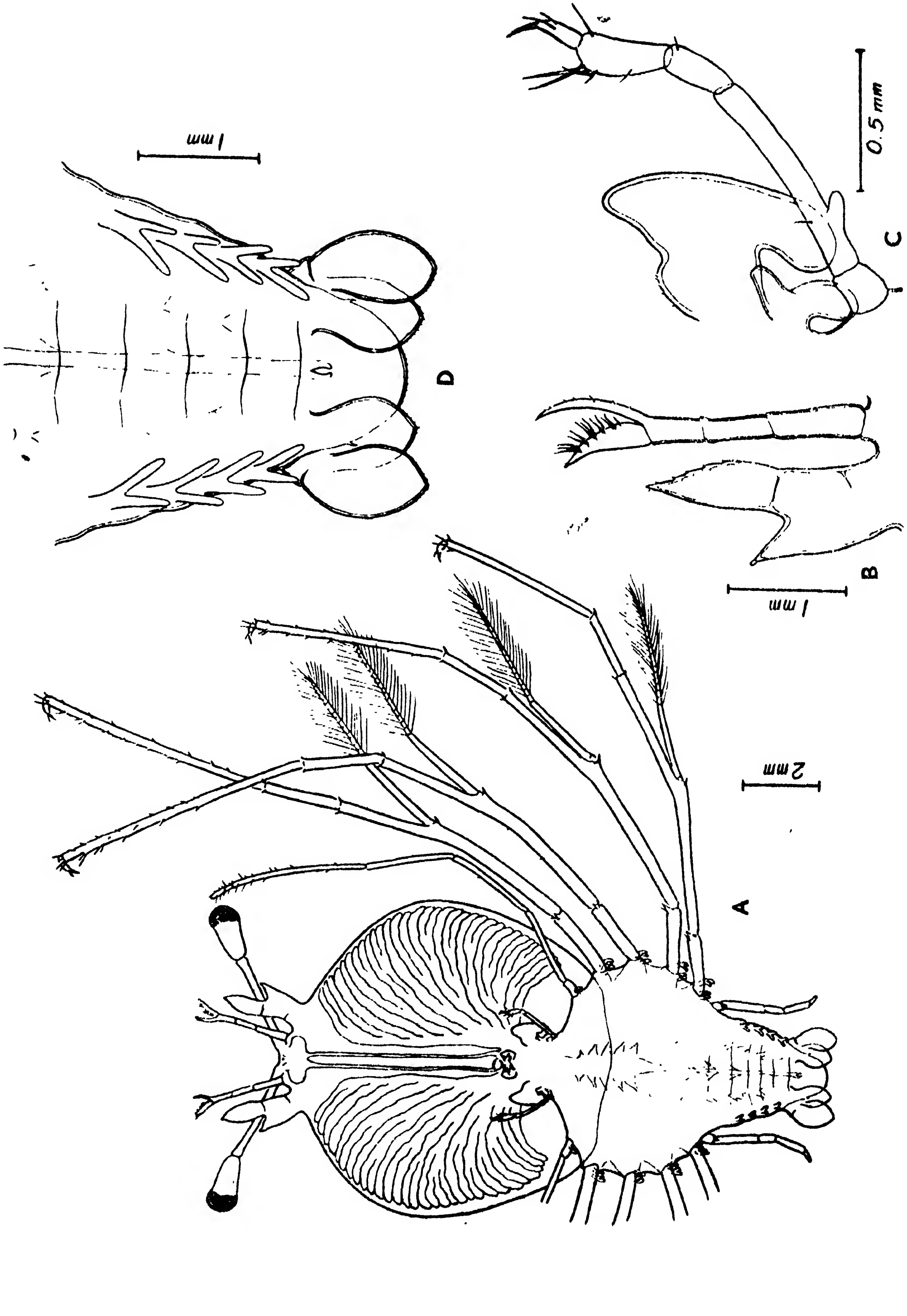
Specimens of *Scyllarus* have not been obtained so far in this area but *S. orientalis* (Spence Bate) has been recorded from the Bay of Bengal and the Arabian Sea and *S. arctus* var. *paradoxus* Miers from the Indian Ocean (Ramadan, 1938). Since *S. orientalis* is common both in the Bay of Bengal and the Arabian Sea the Phyllosoma described here may, in all probability, belong to this species.

Seasonal Distribution of Phyllosoma in the Plankton

Plankton studies at many centres along the coasts of India have shown that Phyllosoma larvae are relatively scarce in the inshore plankton. Aiyar, Menon and Menon (1936) have reported their occurrence in the Madras plankton during December to February. Menon (1945) observed Phyllosoma at intervals during November to March in small numbers off Trivandrum but in January 1939 he noticed swarms of these. Alikunhi (1948) has remarked that these larvae, though not very common, form a conspicuous item of the macroplankton of the Madras

Explanation of the figure 12.

Figure 12. A. Phyllosoma VII of *Scyllarus* sp. B. The first and second antennae. C. The second maxilla, the rudimentary first maxillipede and the second maxillipede. D. The abdomen.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coast particularly during March. In the waters around the Krusadai Island, Chacko (1950) recorded them as occurring during October to February. Bal and Pradhan (1952) recorded their occurrence in Bombay waters during December to April and off Calicut George (1953) observed that they are generally rare except for stray specimens during January to March. Prasad (1954) reported that Phyllosoma were generally found in the plankton of the Mandapam area during February to March and occasionally in June.

In spite of the scarcity of the Phyllosoma larvae in the inshore plankton it is interesting to note that the period of occurrence reported by the various investigators, both along the east and west coasts of India, is almost the same i.e., from December to March or April with the maximum number probably during January to March. Most of the early stages available to the present authors were obtained during January to March and the late stages during June to September. As mentioned earlier an ovigerous *P. ornatus* was obtained in January. A single specimen of *P. fasciatus*, caught on August 31, 1956, moulted in the aquarium on December 26 and on January 30, 1957 it became berried. The berried specimen of *T. orientalis* was, however, caught early in July and the larvae hatched in the aquarium in the third week of July. This may be an instance of off-season spawning which is not uncommon among the decapod crustacea. It seems probable, therefore, that breeding takes place generally from December to March or April and the occurrence of late stages during June to September suggests that the larval life may extend up to about six months. Sheard (1949) observed in Western Australia that in *Panulirus longipes* hatching of eggs takes place from December to February with some isolated cases in November and March while the puerilla and the first juvenile lobsters were seen from June to October and that the duration of larval life in different species ranges from about three to perhaps six or seven months.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. N. Kesava Panikkar for going through the manuscript and suggesting improvements. They also thank Shri S. V. Bapat for placing at their disposal the specimens of Phyllosoma collected by him.

SUMMARY

The first Phyllosoma of *Panulirus ornatus* and *Thenus orientalis* has been described based on larvae hatched in the aquarium. Attempts to rear the larvae in the aquarium to study the complete larval history proved unsuccessful. Descriptions of three more stages of Phyllosoma of *T. orientalis* and seven stages of Phyllosoma of a species of *Scyllarus*, persumably *S. orientalis*, obtained from the local plankton, have been given. From the available data the occurrence of Phyllosoma in the Indian coastal waters appears to be during December to April which gives some indication of the breeding season. The larval life seems to be protracted and may extend up to about six months.

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Issued November 24, 1957.

FURTHER REPORT ON THE FOSSIL MICROFLORA FROM THE MOHGAON KALAN BEDS OF THE MADHYA PRADESH, INDIA

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(Communicated by G. P. Majumdar, F.N.I.)

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INTRODUCTION

This paper deals with further investigation of the fossil microflora from the Deccan Intertrappean Series exposed near the village Mohgaon Kalan in the Chhindwara District of the Madhya Pradesh. In continuation of the previous work (Chitaley, 1947, 1950, 1951, and 1951*a*), more pieces of the promising fossil cherts recently collected from the same locality by the author were macerated with HF for further investigation. Clear dehydrated mounts were prepared by passing the material through different grades of alcohol and finally mounting in canada balsam. Some of the preparations were stained with saffranin and were found to be better for the microscopic examination.

It is of great interest to have found in this investigation new types of angiospermic pollen grains and pteridophytic spores, not observed in the previous maceration. Pollen grains belonging to Betulaceae, Tiliaceae and Ericaceae have been observed in the present examination. Pteridophytic spores are frequent and resemble those of Gleicheniaceae, Lycopodiaceae, and Polypodiaceae.

It has been found difficult to identify correctly without any hesitation spores and grains in fossilised condition due to indistinctness of structure. To overcome such difficulty and to facilitate the work, it is essential to build up a pollen herbarium for comparison. Nevertheless, a good collection of living pollen described in a handy form like the one published by Su Ting (1949) is very useful for quick reference.

The presence of different types of fungal fructifications in the present maceration is noteworthy. Previously too they were reported (Chitaley, 1947, 1950). Some of the present fungal fructifications are indeed interesting in their resemblance to Microthyriaceous fruit bodies, recorded by others from Tertiary beds (Cookson, 1947, Edwards, 1922). Fructifications resembling those of Pleosporaceae, and Mucoraceae have been also discovered in this investigation. A few types not identified are kept under Incertae sedis. Characteristic cuticles have also been found.

AGE OF THE BEDS

The Deccan Intertrappean Series exposed near the village Mohgaon Kalan was described as Tertiary by Sahni (1934, 1940). However, the Geological Survey of India had previously decided the age of this Series as Upper Cretaceous (Blanford 1869, Medlicott and Blanford 1879, and Oldham 1893).

TECHNIQUE

Hydrofluoric acid was used for the maceration of the fossil cherts. The prepared maceration was washed many times with distilled water to remove

all the traces of hydrofluoric acid. The residue was then treated as follows :

50 per cent alcohol	..	..	..	15 minutes
70 per cent alcohol	..	..	..	12 hours
90 per cent alcohol	..	..	..	15 minutes
Absolute alcohol	..	..	..	Over night
Xylol	..	..	..	5 minutes

After this procedure canada balsam mounts were prepared in the following way :

Thin canada balsam was taken in a small watch glass and the residue from the xylol was transferred to it. After stirring well a drop of the paste was taken on the slide and was spread in a thin film and covered with a cover slip. The preparations prepared with such technique were found to be very clear and devoid of any air bubbles for the microscopic examination. Every possible care was taken to avoid any foreign contamination.

Classification and Nomenclature

For keeping up the continuity with the previous paper in this series (Chitaley 1951*a*), the classification and terminology have been kept the same adopted from the system introduced by Erdtman (1943 and 1947).

Description

The following spores and pollen grains are all measured in μ.

Pteridophytic spores :

Alites

Alites spm. :—(Text-fig. 1, A). Spore spherical, tetrad scar absent: the surface of the spore with thick warts ; 28 μ.

Alites spm. :—(Text-fig. 1, B). Spore spheroidal, tetrad scar not seen ; exine thick, pilatus ; 22 μ.

Monolites

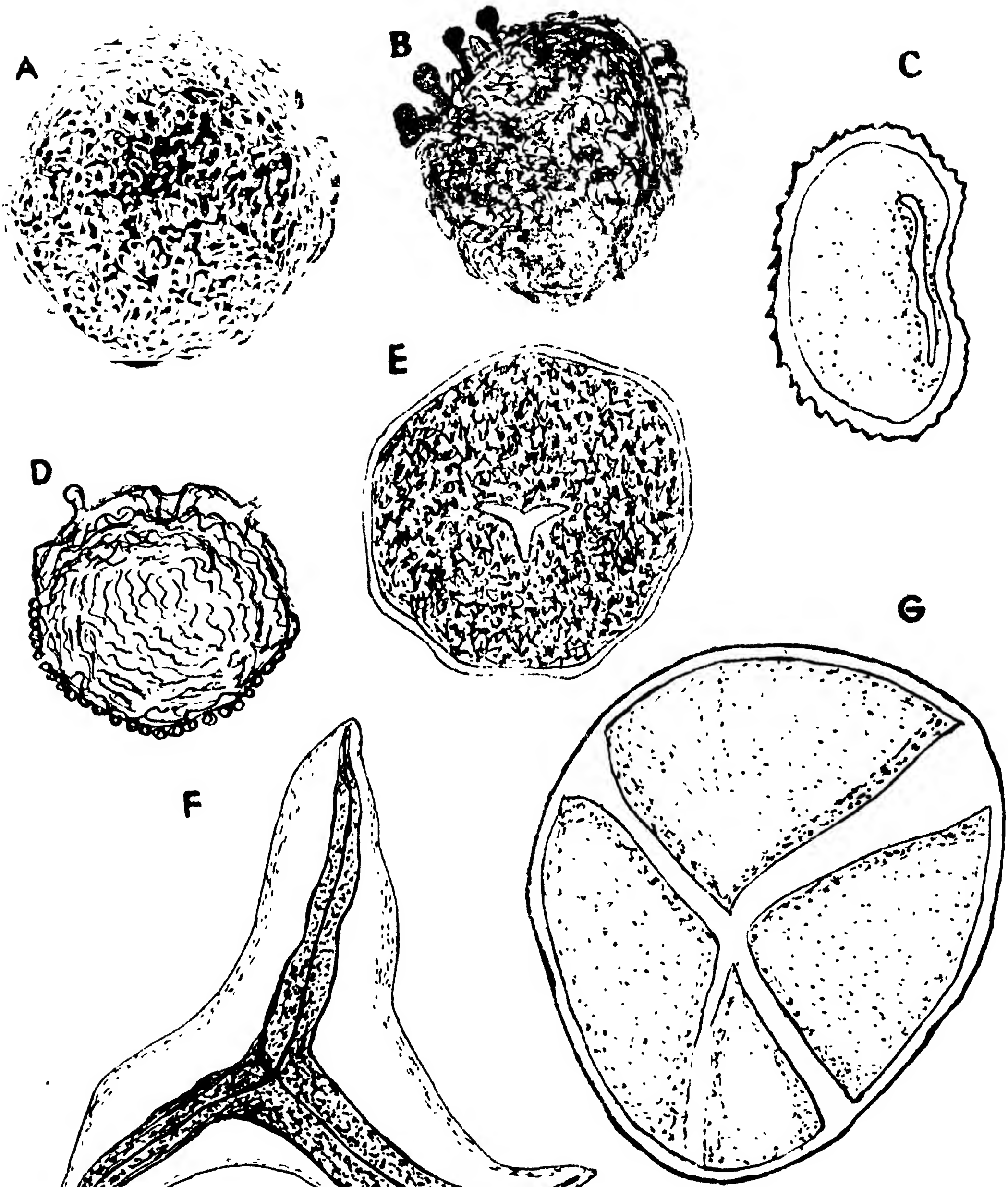
Monolites (*Polypodites*) *spm.* :—(Text-fig. 1, C). Spore concavo convex, monolite, with rough perine present; exposed in lateral view : 28 × 18 μ.

Monolites spm. :—(Text-fig. 1, D). Spore spherical, monolite scar shown on the upper side of the figure : spore wall hairy ; 24 μ.

Monolite spores are generally found in Polypodiaceae (Selling 1946, Naumova 1937, and Erdtman 1943). Potonie and his co-workers (1950) have also described them from miocene and Thiergart (1950) has shown their presence in Tertiary beds. A polypodiaceous naked spore without a perine was described in the previous paper (Chitaley 1951*a*). The present specimen seems to be a spore with perine and may belong to Polypodiaceae.

Triletes

Triletes (*Lycopodites*) *spm.* :—(Text-fig. 1, E). Spore spherical, with thick warty wall ; tetrad scar present ; this type bears a superficial resemblance to that of Lycopodiaceae. Lycopodiaceous spores have been frequently recorded from Tertiary beds (Potonie *et al.*, 1950) ; 33 μ.



TEXT-FIG. 1 :—All figures from A to G are × 1.[illegible].

Pteridophytic Spores.

A. *Alites spm.*
B. *Alites spm.*
C. *Monolites (Polypodites) spm.* Lateral view.
D. *Monolites spm.*
E. *Triletes (Lycopodites) spm.*
F. *Triletes (Gleichenidites) spm.* Triradiate mark prominent.
G. *Triletes spm.* Tetrad scar present.

Triletes (*Gleichenidites*) *spm.* :—(Text-fig. 1, F). Spore triangular, with concave sides and attenuated corners. The triradiate mark is prominent with prominent ridges ; spore wall thick and smooth : 48 μ.

The present spore bears a close resemblance to that of Gleicheniaceae. Nils-Erik Ross (1949) has shown its presence in the Upper Cretaceous beds of Scania, and Leschik (1955) has reported it from Tertiary beds of Basel. He is not sure about its affinity to Gleicheniaceae.

Triletes spm. :—(Text-fig. 1, G). Spore spheroidal, with prominent tetrad scar, with broad ridges extending and broadening to the periphery; wall of the spore smooth and thick : 42 μ.

Angiospermic pollen grains :

A number of pollen grains are obtained in the present investigation of the microfloral material. They are of different types. A few of them belong to the monocotyledonous group and the rest are all dicotyledonous.

Monocotyledoneae

Monosulcites spm. :—(Text-fig. 2, H). Pollen grains monosulcate, oblong or boat shaped, with single broad sulcus reaching from end to end: exine thin and smooth ; 24×12 μ.

Monoporites (*Graminidites*) *spm.* :—(Text-fig. 2, I). Pollen grains minute, each one is spherical, with thin smooth wall and one germinal pore surrounded by a poral rim which is approximately 1 μ in width : size of the grain 12 μ.

Pollen grains belonging to Gramineae were described in the author's previous paper. These grains also may belong to the same family, though differing in size and shape.

Dicotyledoneae

Monocolpites spm. :—(Text-fig. 2, J). Grain monocolpate, oblique equatorial view; elliptical, with thick psilate wall : the colpa extending from end to end. 36×16 μ.

Tricolpites (*Ericacidites*) *spm.* :—(Text-fig. 2, K). Grains in rhomboidal tetrad ; exine thick with more or less uneven surface ; size of the tetrad, $32 \times 28\mu$. The grains of Ericaceae are often found in rhomboidal tetrads particularly in the genus *Calluna* ; the present tetrad is very much similar to an Ericaceous one (Erdtman 1943). The importance of the presence of an Ericaceous tetrad in

EXPLANATION OF TEXT-FIGURES

TEXT-FIG. 2 :—All figures from H to T are $\times$ 1200.

Monocotyledonous Pollen Grains.

H. *Monosulcites spm.*

I. *Monoporites* (*Graminidites*) *spm.*

Dicotyledonous Pollen Grains.

J. *Monocolpites spm.*

K. *Tricolpites* (*Ericacidites*) *spm.* Rhomboidal tetrad.

L. *Tricolpites spm.* Polar view.

M. *Tricolpites spm.* Equatorial view.

N. *Tricolporites* (*Tiliacidites*) *spm.* Polar view.

O. *Tricolporites spm.* Oblique equatorial view.

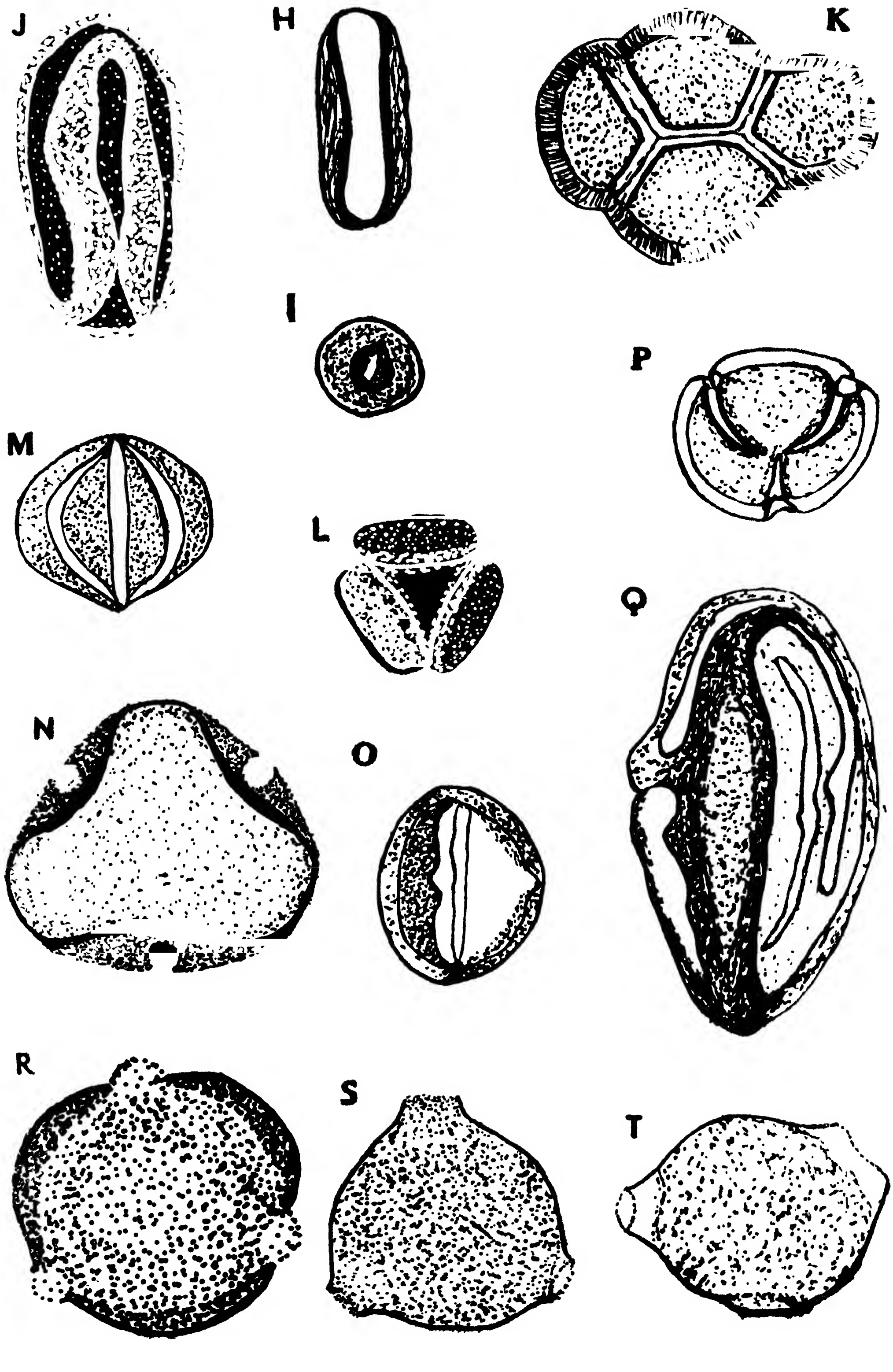
P. *Tricolporites spm.* Polar view.

Q. *Tricolporites spm.* Equatorial view.

R. *Triorites spm.*

S. *Triorites spm.*

T. *Triorites spm.*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the present beds can not be ignored. However, Ericaceous grains are very rarely recorded in eocene age and according to Potonie (1951) they have not been reported so far from any of the pre-eocene beds. They have been frequently recorded from Oligocene and Miocene.

Tricolpites spm. :—(Text-fig. 2. L) Grain tricolpate, polar view; wall thin and psilate; 18μ.

Tricolpites spm. :—(Text-fig. 2. M). Grain tricolpate, equatorial view, more flattened in breadth, furrows clear; wall thin psilatus; 20μ.

Tricolporites (*Tiliacidites*) *spm.* :—(Text-fig. 2, N). Grain tricolporate, polar view, faintly triangular; the surface of the grain is rough, furrows short, with appendant germ pores; under an ectexine, there is a mesexine filling. Size 32μ.

The present grain shows much resemblance to a *Tilia* grain described by Kirchheimer (1932).

Tricolporites spm. :—(Text-fig. 2, O). Grain tricolporate, oblique equatorial view; spheroidal; wall thick and psilate, germinal pores present in the furrows; 20×16μ.

Tricolporites spm. :—(Text-fig. 2. P). Grain tricolporate, psilate, furrows deep, narrowing to the centre, germinal pores distinct, one in each furrow; exposed in polar view; 22μ.

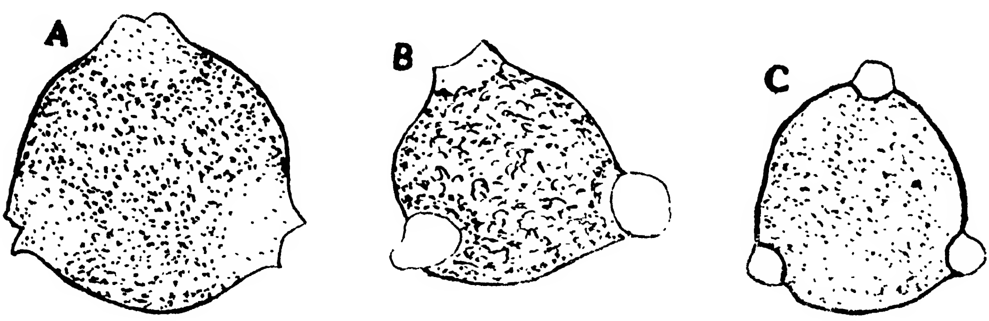
Tricolporites spm. :—(Text-fig. 2. Q). Grain tricolporate, exposed in oblique equatorial view, ovoidal, greater in length than in breadth; wall thick, psilatus, furrows long with germinal pores; 44×24μ.

Triorites spm. :—(Text-fig. 2, R). Grain triorate, spherical, psilate; exine thick of approximately 1μ in width, the pores buldging out; the size of the grain 30μ.

Triorites spm. :—(Text-fig. 2, S). Grain triorate, arci sharp, pores non-aspidate, simple; surface of the grain reticulate, 24μ.

Triorites spm. :—(Text-fig. 2, T). Grain triorate, ores on the two lateral sides are clear; spheroidal; psilate; 24×22μ.

Triorites (*Betulacidites*) *spm.* :—(Text-fig. 3, A). Grain spherical, exposed in polar view, triorate; exine thin, psilate, arci fairly well developed, with three non aspidate pores; 28μ.



TEXT-FIG. 3 :—*Dicotyledonous Pollen Grains.*

A. *Triorites* (*Betulacidites*) *spm.* Polar view. × 1200.
B. *Triorites* (*Betulacidites*) *spm.* Oblique Polar view. × 1200.
C. *Triorites* (*Betulacidites*) *spm.* Pores aspidate, buldging out. × 1200.

The grain of *Carpinus*, a genus belonging to Betulaceae, very much resembles the present fossil grain.

Triorites (*Betulacidites*) *spm.* :—(Text-fig. 3, B). Grain triorate; exine thin and warty; the two germinal pores bulging out, pores aspidate, arci clear; oblique polar view of the grain; 24μ.

Triorites (*Betulacidites*) *spm.* :—(Text-fig. 3, C). Grain spherical, triorate; pores aspidate, bulging out, arci sharp, exine smooth, surface psilate; 22μ.

Fungi :

Different kinds of fungal fructifications have been also observed in the present maceration. For convenience they are described as type 1, type 2, and so on.

Type 1 :—(Text-fig. 4, A and B). This is a shield shaped fruit body with radial structure, dark brown in colour. The margin of the body is sinuous; each fruit body consists of number of slender hyphae, radially arranged; the cells are cubical, dense in the centre, stoma absent; one or two mycelial hyphae are seen attached to the body in the present specimen. Size of the fruit body about 130μ in diameter.

This specimen bears a close resemblance to the fruit bodies of the sub family Asterineae of the Microthyriaceae fungus. Although the present specimen is lacking in complete mycelium, yet its remains are clearly seen attached to the fruit body. It is invariably very difficult to get complete mycelium in fossilised condition when the fruit bodies are not found on the cuticles.

This type of Microthyriaceous ascomata has been already described by Edwards (1922) from Eocene beds and by Cookson (1947) from Oligo-Miocene beds.

Type 2 :—(Text-fig. 4, E). Stalked multicellular fruit bodies have been also observed. Each body is composed of 14–15 cells. Size of each fructification is 75μ in length without stalk and the size of the stalk is 37μ in length. This type of fructification shows a superficial resemblance to that of *Alternaria* or *Rivularia* type of fungus.

Type 3 :—(Text-fig. 4, H and I). Fungal fruit bodies are frequently met with in the maceration. Formation of a zygospore by the fusion of the two different hyphae is obvious though the gametangial ends alone are seen to be present. It looks as if the zygospores are broken off with the gametangial ends and a few cells, from the main hyphae. The zygospore is round and its contents are darker than those of the gametangial ends of the hyphae. Size of the zygospore about 15μ, width of each gametangial end about 9μ. The hyphae which bear gametangia are septate. These fructifications very much resemble those of Mucoraceae except the septate nature of the hypae which is very much unlikely to be found in Mucoraceae.

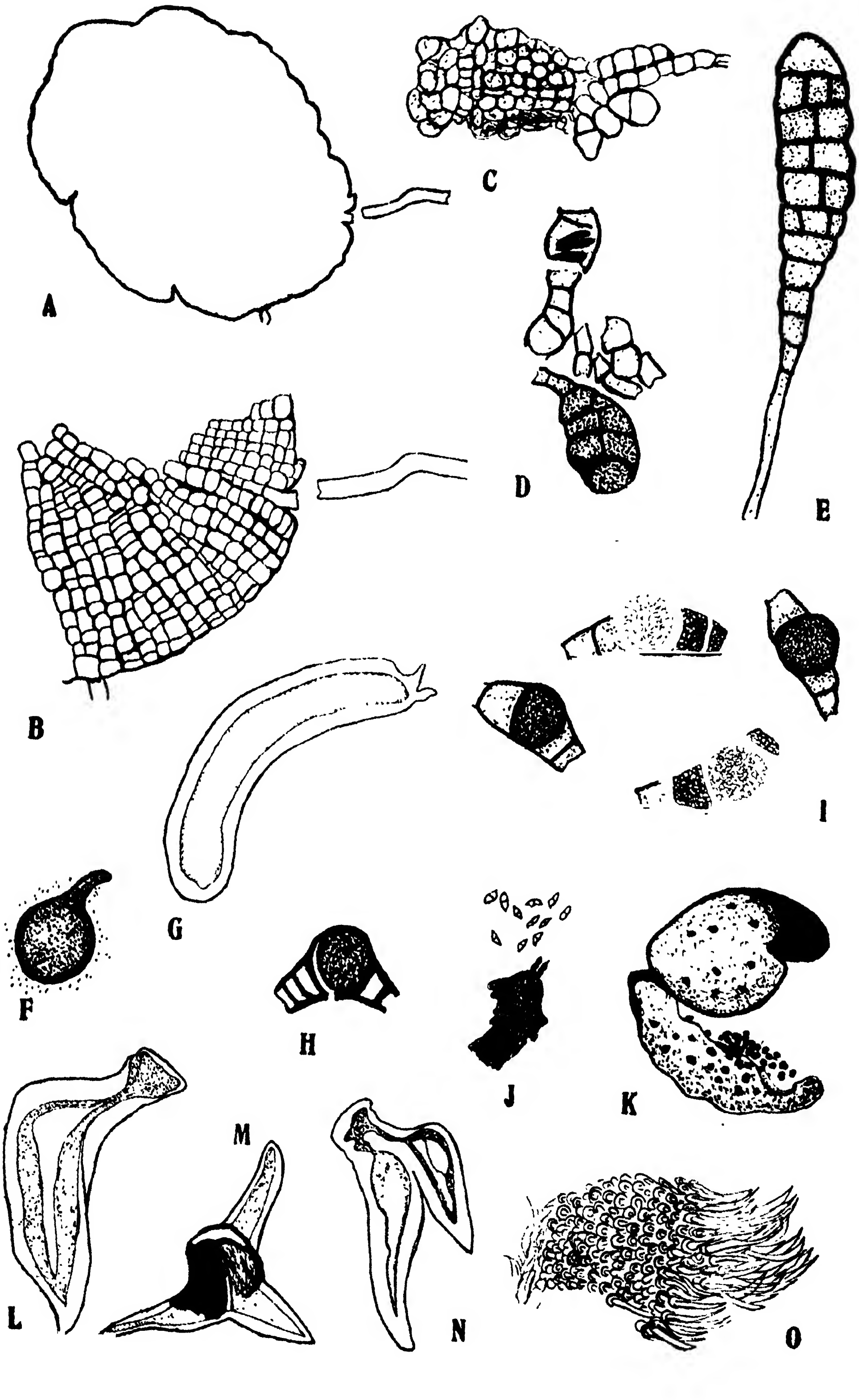
Type 4 :—(Text-fig. 4, D). A number of fungal hyphae are found together. Each one is septate, and breadth of each cell is about 6μ. Interspersed with these hyphae are stalked fruit bodies. Each body is multicellular and 25μ in length.

These fructifications look very much like the muriform spores of the Pleosporaceae, a family of Ascomycetes.

Incertae sedis

Type 5 :—(Text-fig. 4, C). Septate hyphae with small cells have been observed with bicellular bodies intermingled. Size of the cell 5μ. Size of the fruit body in length 10μ.

Type 6 :—(Text-fig. 4, G). Another specimen is a long and narrow small cucumber like structure. The tip is rounded, wall thick and smooth. Size 80μ in length.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Type 7 :—(Text-fig. 4, J). Bicellular minute bodies attenuated at both ends have been grouped together near a number of hyphae which are vertically arranaged in a bundle. The bicellular bodies seem to have come out from these hyphae.

Type 8 :—(Text-fig. 4, F). It is a stalked round body brown in colour, probably a fungus. The stalk is slightly curved. The wall is thick and smooth. The diameter of the body is 26μ and the length of the stalk is about 13μ.

Type 9 :—(Text-fig. 4, K). This is a dark brown spherical body measuring about 50μ in diameter. It looks very much like a dehisced sporangium liberating a mass of spores. The wall at the line of fracture seems to have more thinned down. Nothing can be definitely said since the cell structure is indistinct and the structure of the spores is obscure.

Cuticles

A well preserved cuticle has been also observed in the investigation with the scars of the fallen off hairs. A few hairs are seen still attached. (Text-fig. 4, O). Two types of simple hairs and one type of peltate hair have also been noted. (Text-fig. 4, L, M, and N).

Discussion

The investigation of the fossil microflora from the Mohgaon Kalan beds of the Madhya Pradesh, India, has been recently started by the author (Chitaley, 1947. 1950 and 1951). The present work is in continuation of the previous investigation, and describes more angiospermic grains and fungal fructifications.

Some of the Betulaceae (Erdtman 1951, and Terasmae 1951) grains have been described in the present paper. All of them although belonging to the same family Betulaceae, differ from each other in details which suggest the presence of different species. Identification of these could not be done due to indistinctness of structures in the fossil grains. Pollen grains resembling those of Tiliaceae and Ericaceae have also been observed in this investigation. They have been elsewhere found to be of great importance in determining the age of the bed. The pollen grains of these families have been reported from Tertiary beds by Potonie and Co-workers (1950) and by Kirchheimar (1932). According to Potonie (1951) these grains predominate in late tertiary period, and are found to be very rare in early tertiary. To the author's knowledge they are not recorded so far in the pre-eocene period. Pollen grains resembling those of Compositae were already reported from the same bed in the previous paper (Chitaley

Explanation of Text-figures

Text-fig. 4 :—

A. *Type 1*, Microthyriaceous fruit body; outline shown × 350.
B. *Type 1*, Portion of the above fruit body magnified showing structural details. × 600.
C. *Type 5*, Incertae sedis. × 600.
D. *Type 4*, Fructifications of ? Pleosporaceae. × 600.
E. *Type 2*, Stalked fruit body of ? *Alternaria* or ? *Rivularia*!fungus. × 600.
F. *Type 8*, Incrotae sedis. × 600.
G. *Type 6*, Incertae sedis. × 600.
H. *Type 3*, Fruit body like ? Mucoraceous zygospore. × 600.
I. *Type 3*, Fruit bodies ? Mucoraceae, in group. × 600.
J. *Type 7*, Incertae sedis. × 600.
K. *Type 9*, Incertae sedis. × 600.
L, M, N, & O, Cuticles. × 600.

1951*a*). These grains too seem to be restricted to tertiary era. They have not been recorded so far in the earlier periods (Potonie 1951).

Pollen grains of Tiliaceae, Compositae and Ericaceae and fungal fructifications of Microthyriaceae discovered in this investigation have been recorded elsewhere from Tertiary beds (Edwards 1922, and Cookson 1947). They are not recorded to the author's knowledge from pre-tertiary beds. The pollen grains of Myrtaceae, Rosaceae, and Betulaceae, and the spores of Gleicheniaceae, Polypodiaceae, and Lycopodiaceae discovered in these beds have been recorded so far from both Cretaceous and Tertiary beds. It is interesting to note that no particular type of spores and grains is in predominance in the present beds of Mohgaon Kalan of the Deccan Intertrappean Series. Pollen grains and spores recovered from both Cretaceous and Tertiary beds as above have been observed mixed up together. These facts give a vivid picture of the type of the plants growing here in olden times. Both Cretaceous and Tertiary flora seem to have existed side by side. However, the knowledge of the Microflora of the Deccan Intertrappean Beds is too meagre to make any definite statement at this stage of the investigation. Further macerations may show more types of grains and spores and more grains of one and the same type to throw further light on the age of the Deccan Traps.

Recently Rao and Vimal (1955) have drawn a comparison between microflora of the Tertiary lignites from different localities and the microflora of the Deccan Intertrappean Series (Chitaley 1951, 1951*a*), which they have taken as of Eocene age. It is evident from the comparison that types like *monocolpites*, *triorites* (? *Betula*) *triletes*, are common in both rocks. Also the presence of *Microthyriaceous* fungus in both Tertiary lignites and the Deccan Intertrappean Series is significant. From the present investigation the author would add that the grain of ? *Carpinus* common in Tertiary lignites is also represented in Deccan Intertrappeans. Tiliaceous grains too are represented in both, although the grains present in lignites are different in being *tetracolporites*, and those present in Deccan Intertrappeans being *tricolporites*. Tiliaceae have both these types of grains in different genera. However, more information on the microflora of the Deccan Intertrappean rocks is essential to draw a detailed comparison of the two floras. Similarly, study of the microflora from Indian Cretaceous beds would be helpful for such comparison. Further investigation on both these beds is being carried out by the author at the Government College of Science, Nagpur.

Summary

This paper deals with the investigation of the Fossil Microflora of the Deccan Intertrappean Beds, exposed near the village Mohgaon Kalan, Chhindwara District of the Madhya Pradesh. The investigation of the macerated chert has revealed new Monocotyledonous and Dicotyledonous grains not previously recorded from these beds. The grains of Betulaceae, Tiliaceae, and Ericaceae are worth mentioning. Pteridophytic spores belonging to Gleicheniaceae, Lycopodiaceae, and Polypodiaceae are frequent in the present maceration. Fungal fructifications of Microthyriaceous type and fruit bodies resembling those of Pleosporaceae and Mucoraceae have been also found in these cherts.

It is interesting to note that the pollen grains, spores and fungal fructifications typical of Tertiary age are represented in the present beds in addition to those recorded from both Cretaceous and Tertiary age.

Further investigation of the Microflora of these Deccan Intertrappean beds from Mohgaon Kalan is being carried out, for collection of data which may prove helpful in deciding the age of these beds on the basis of microfloral analysis.

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A CONTRIBUTION TO THE KNOWLEDGE OF THE DIATOMACEAE OF KANYA KUMARI (CAPE COMORIN), INDIA—I

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(Communicated by M. S. Randhawa, F.N.I.)

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Menon (1931), Iyer *et al.* (1936), Menon (1945) and Subrahmanyan (1946) have recorded the Marine Diatoms from the East Coast and Misra (1956) from the West Coast. There is no record of the Marine Diatoms from the coast of Kanya Kumari, the southernmost point of the Indian peninsula. The present communication is based on the collections from this region during June 1956.

In all 29 forms have been described in this paper representing 16 genera. Of the total forms described three represent new varieties and two new forms.

SYSTEMATIC ENUMERATION

1. *Melosira granulata* (Éhr) Ralfs. var. *anguistissima* Müll. Hustedt, 1930*b*, Bd. VII, Teil 1, p. 250, Fig. 104d; Ventakaraman, 1939, p. 299, Fig. 2.

Frustules linked in long chains with narrow and long cells (Text-figs. 3 and 4).
Diam. frustule, 3.8—5.7μ.
Height of the cell, 11.4—15.2μ.
Punctae, 10—12 in 10μ.
Habitat: Planktonic. 14th June, 1956.

2. *Terpsinoe musica* Éhr. Van Heurck, 1899, p. 452, Fig. 176; Venkataraman, *op. cit.*, p. 301, Figs. 18–21.

Frustules quadrangular in girdle view. Valves linear with undulating margins, slightly knobbed at the extremities. Frustules divided into 6–7 parts by cross septa. Coarsely punctate (Text-fig. 1).
Diam. frustules, 35—45.6μ.
Length frustules, 95—144.4μ.
Punctae, 8—10 in 10μ.
Habitat: Epiphytic on *Chaetomorpha* sp. 14th June 1956.

3. *Synedra ulna* (Nitz) Éhr. var. *oxyrhyncus* (Kütz) Heurck. Heurck, *op. cit.*, p. 331, Fig. 418; Venkataraman, *op. cit.*, p. 307, Fig. 38.

Frustules linear, delicately and closely striated (Text-fig. 11).
Breadth frustule, 7.6μ.
Length frustule, 72.2μ.
Habitat: On the bottom mud of some saline pools. 14th June, 1956.

4. *Synedra ulna* (Nitz) Éhr. var. *crassa* var. nov.

Valves linear, strongly constricted in the middle, ends wedge-shaped, pseudoraphe narrow and linear, striae absent in the central area (Text-fig. 13).
Breadth frustule, 7.6μ.
Length frustule, 110.4μ.
Striae, 12—14 in 10μ.
Habitat: On the bottom mud of some saline pools, 14th June, 1956.

This form differs from *S. ulna* var. *constricta* Venkat, in its bigger frustules and its slightly wedge shaped ends.

5. *Synedra ulna* (Nitz) Éhr. var. *amphirhyncus* (Éhr) Grün. Hustedt, *op. cit.*, p. 200, Fig. 619A, *e*; Venkataraman, *op. cit.*, p. 308, Figs. 28, 30, 31, 32.

Valves linear, lanceolate, broad nearly to the end. Thee entral area shows much variation viz. complete absence of striae or the striae being shorter on one side and absent on the other etc. (Text-fig. 12).

Breadth frustule, 7.6μ.
Length frustule, 220.4μ.
Striae, 10—12 in 10μ.
Habitat: Planktonic. 15th June, 1956.

6. *Enuotia monodon* Éhr. Hustedt, *op. cit.*, p. 305, Fig. 772*a*, *b*; Venkataraman, *op. cit.*, p. 310. Fig. 40.

Valves arcuate, ends rounded striations coarse (Text-fig. 2).
Breadth frustule, 7.6 —11.4μ.
Length frustule, 26.6 —72.2μ.
Striae, 8—10 in 10μ.
Habitat: Bottom mud of saline pools. 14th June. 1956.

7. *Gyrosigma balticum* (Éhr) Rabh. Hustedt, *op. cit.*, p. 224, Fig. 331; Venkataraman, *op. cit.*, p. 318, Figs. 71, 72.

Valves slightly sigmoid, broad, raphe eccentric and slightly sigmoid (Text-fig. 21).
Breadth frustule, 30.4—34.2μ.
Length frustule, 209—323μ.
Striae, 12—15 in 10μ.
Habitat: Planktonic. 15th June, 1956.

8. *Pleurosigma galapagense* Cleve var. *kumariensis* var. nov.

Valves scarcely sigmoid, lanceolate, tapering from the middle. Raphe slightly sigmoid (Text-fig. 22).

Breadth frustule, 30.4μ.
Length frustule, 152μ.
Oblique striae, 22 in 10μ.
Transverse striae, 24 in 10μ.
Habitat: Planktonic. 15th June, 1956.

This form differs from the type in its robust cells and slightly greater number of oblique and transverse striae.

9. *Pleurosigma Spencerii* var. *curvata* Grün. Van Heurck, *op. cit.*

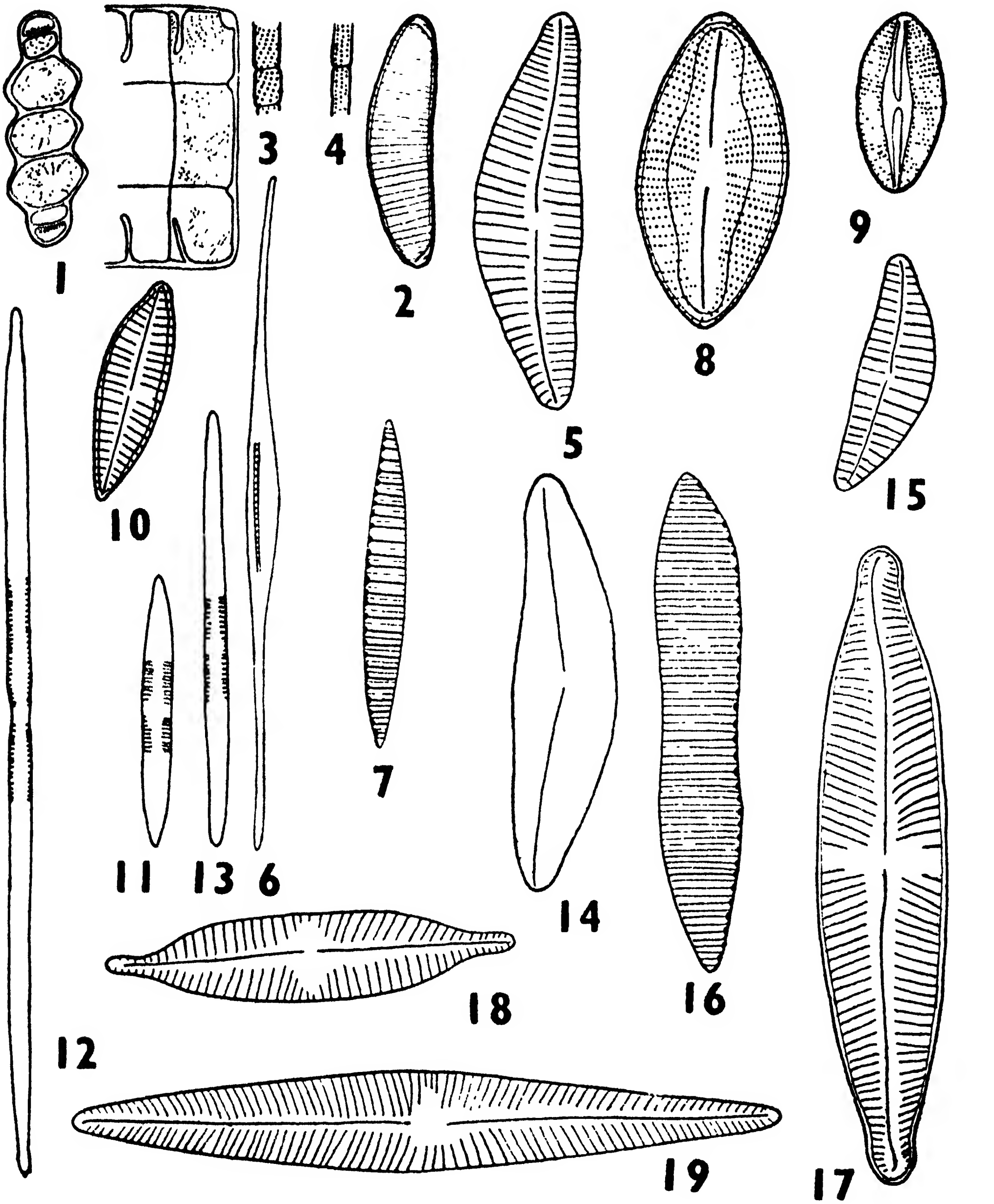
Valves sigmoid, raphe slightly eccentric and sigmoid (Text-fig. 25).
Breadth frustule, 7.6μ.
Length frustule, 60.8μ.
Longitudinal striae, 8—10 in 10μ.
Transverse striae, 10—15 in 10μ.
Habitat: Bottom living in some saline pools. 14th June, 1956.

10 *Navicula fusca* var. *hyperborea* Heurck. Van Heurck, *op. cit.*, p. 199, Pl. 26, Fig. 745.

Valves elliptical with clearly punctate striae (Text-fig. 8).
Breadth frustule, 13.2μ.
Length frustule, 32.3μ.
Striae, 7—8 in 10μ.
Habitat: Bottom living in some saline pools. 14th June, 1956.

11. *Navicula elliptica* var. *ovalis* Hilse. Van Heurck, *op. cit.*

Valves elliptical with punctate striae (Text-fig. 9).
Breadth frustule, 11.4μ.
Length frustule, 26.6μ.
Striae, 12—13 in 10μ.
Habitat: Free-living. 15th June, 1956.



TEXT FIGS. 1–19.—Fig. 1. *Terpsinoe musica* Ehr. Fig. 2. *Eunotia monodon* Ehr. Figs. 3 & 4. *Melosira granulata* (Ehr) Ralfs. var *anguistissima* Müll. Fig. 5 & 15. *Cymbella turgida* (Greg.) Cleve. Fig. 6. *Nitzschia longissima* (Bréb) Ralfs. Fig. 7. *Nitzschia palea* (Kütz) W. Smith. Fig. 8. *Navicula fusca* var. *hyperborea* Heurck. Fig. 9. *Navicula elliptica* var. *ovalis* Hilse. Fig. 10. *Gomphonema parvalum* var. *subcapitata* Heurck. Fig. 11. *Synedra ulna* var. *oxyrhyncus* (Kütz) Heurck. Fig. 12. *Synedra ulna* var. *amphirhyncus* (Ehr) Grün. Fig. 13. *Synedra ulna* var *crassa* var. nov. Fig. 14. *Cymbella cistula* var. *maculata* (Kütz) Heurck. Fig. 16. *Nitzschia tryblionella* var. *levidensis* (W. Smith) Grün. Fig. 17. *Pinnularia interrupta* f. *genuina* Fritsch. Fig. 18. *Navicula halophila* f. *subcapitata* Östrup. Fig. 19. *Navicula hasta* Panto. (Fig. 1, × 540; 2,5,7–10,14–19, × 1600; 3,4,6,11,12, and 13, × 685).

12. *Navicula halophila* f. *subcapitata* Östrup. Hustedt, 1930*a*, Heft 10, p. 269; Venkataraman, *op. cit.*, p. 327, Fig. 91.

Valves with slightly produced and capitate ends. Central area slightly widened in the middle (Text-fig. 18).

Breadth frustule, 7.6μ.
Length frustule, 38μ.
Striae, 9 in 10μ.
Habitat: Free-living. 15th June, 1956.

13. *Navicula hasta* Pantocsek. Hustedt, 1930*a*, p. 306, Fig. 541; Venkataraman, *op. cit.*, p. 331, Fig. 98.

Valves lanceolate, gradually tapering to the ends; ends subacute, striations radial (Text-fig. 19).

Breadth frustule, 11.4μ.
Length frustule, 60.2μ.
Striae, 9—10 in 10μ.
Habitat: Bottom living in saline pools. 16th June, 1956.

14. *Navicula cuspidata* Kütz var. *conspicua* Venkataraman. forma *crassa* form nov.

Valves elliptic, lanceolate with rounded slightly constricted ends. Longitudinal striations coarse and prominent, closer towards the margins and wider towards the middle (Text-fig. 26).

Breadth frustule, 30.4—38μ.
Length frustule, 129.2—163.4μ.
Transverse striae 18—20 in 10μ.
Longitudinal striae 8—16 in 10μ.
Habitat: Bottom living in saline pools. 16th June, 1956.

This form differs from the variety in slightly bigger dimensions and in greater number of the transverse striae.

15. *Navicula cuspidata* Kütz. var. *ambigua* (Éhr) Cleve. Hustedt, 1930*b*, p. 268, Fig. 434; Venkataraman, *op. cit.*, p. 327, Fig. 94.

Valves elliptic, lanceolate with rostrate produced ends. Longitudinal striae are equally placed (Text-fig. 29).

Breadth frustule, 22.8μ.
Length frustule, 72.2μ.
Transverse striae, 20 in 10μ.
Habitat: Bottom living in saline pools. 17th June, 1956.
This form has slightly boarder frustules than the type.

16. *Pinnularia interrupta* W. Smith. f. *genuina* Fritsch. Venkataraman, *op. cit.*, p. 336, Fig. 112.

This form shows interruption of striae at the middle of the valve (Text-fig. 17).
Breadth frustule, 11.4μ.
Length frustule, 76μ.
Striae, 10 in 10μ.
Habitat: Bottom living in saline pools. 16th June, 1956.

17. *Pinnularia viridis* (Nitz) Éhr. Hustedt, 1930*b*, p. 334, Fig. 617*a*; Venkataraman, *op. cit.*, p. 339, Fig. 114.

Valves linear with slightly convex margins and rounded ends. Axial area narrow, slightly widened at the middle, striae coarse. The longitudinal band present (Text-fig. 27).

Breadth frustule, 19μ.
Length frustule, 72.2μ.
Striae, 8—9 in 10μ.
Habitat: Bottom living in the crevice of rocks. 16th June, 1956.

18. *Amphora coffoeoformis* Agardh. var. *africana* Fritsch and Rich. Venkataraman, *op. cit.*, p. 342, Fig. 102.

Forma *kurze* form nov.

Valves arcuate dorsally and straight ventrally, ends pronouncedly capitate, striae delicately punctate (Text-fig. 31).

Breadth frustule, 4.7—7.6μ.

Length frustule, 22.8—38μ.

Striae, 18—22 in 10μ.

Habitat: Free-living. 17th June, 1956.

This form differs from the type in its greater number of striae.

19. *Cymbella turgida* (Greg.) Cleve. Hustedt, 1930*b*, p. 358. Fig. 660; Venkataraman, *op. cit.*, p. 343, Fig. 125.

Valves luneate with convex dorsal side and gibbous ventral margin. Ends acute, striations radial in the middle and slightly convergent towards the ends, punctate (Text-figs. 5 and 15).

Breadth frustule, 11.4μ.

Length frustule, 22.8 - 34.2μ.

Striae, 8 - 9 in 10μ.

Habitat: Free-living. 16th June, 1956.

20. *Cymbella cistula* (Hemp) Grün. var. *maculata* (Kütz) Heurck. Van Heurck, *op. cit.*, p. 147, Pl. 1, Fig. 41; Venkataraman, *op. cit.*, p. 344, Fig. 136.

Frustules boat-shaped with ventral gibbose margins and rounded ends, striations radial, punctate (Text-fig. 14).

Breadth frustule, 30.4μ.

Length frustule, 110.2μ.

Striae, 8—10 in 10μ.

Habitat: Free-living. 17th June, 1956.

21. *Gomphonema parvulum* (Kütz) Grün. var. *subcapitata* Heurck. Van Heurck, *op. cit.*, p. 272.

Valves club-shaped with subcapitate end (Text-fig. 10).

Breadth frustule, 7.6μ.

Length frustule, 22.8μ.

Striae, 12—13 in 10μ.

Habitat: Epiphytic on *Chaetomorpha* sp. 15th June, 1956.

22. *Rhopalodia gibba* (Éhr) O. Müll. Venkataraman, *op. cit.*, p. 349, Fig. 115.

Frustules gibbous in the middle, slightly tapering at the ends, reflexed at the ends, costae strong.

Breadth frustule, 22.8—26.6μ.

Costae, 6—8 in 10μ.

Habitat: Bottom living in saline pools. 16th June, 1956.

23. *Hantzschia amphioxis* (Éhr) Grün. var. *vivax* (Hantz.) Grün. Van Heurck, *op. cit.*, p. 381, Pl. 15, Fig. 486b; Venkataraman, *op. cit.*, p. 351, Fig. 148.

Valves linear, slender, keel punctae short (Text-fig. 30).

Breadth frustule, 7.6μ.

Length frustule, 60.8μ.

Keel punctae. 8 in 10μ.

Striae, 16 in 10μ.

Habitat: From the scrapings of the rocks. 17th June, 1956.

24. *Bacillaria paradoxa* Gmelin. Venkataraman, *op. cit.*, p. 351, Figs. 144 and 145; Subrahmanyan, 1946, p. 187, Figs. 417, 421 and 427; Misra, 1956, p. 566, Fig. 71.

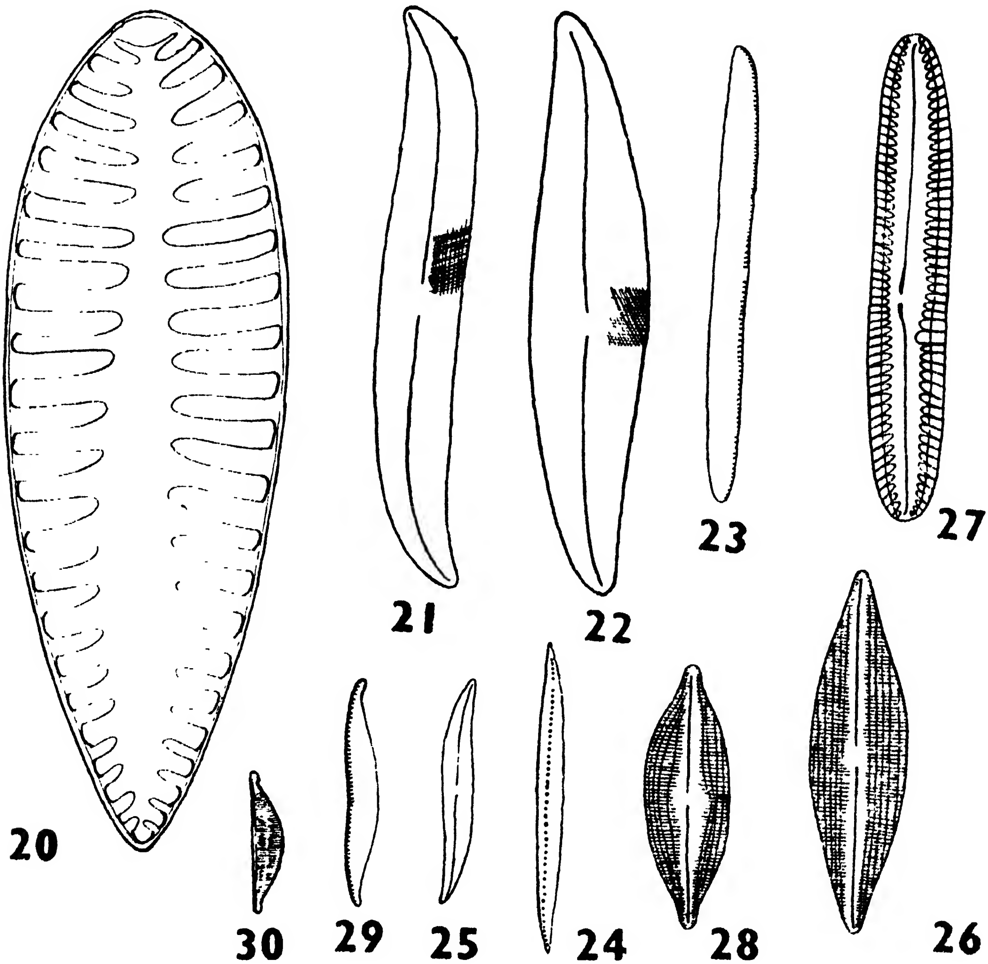
Valves linear, spindle shaped, carinal dots form a row in the middle portion of the valve (Text-fig. 24).

Breadth frustule, 5.7μ.
Length frustule, 76μ.
Carinal dots, 8—10 in 10μ.
Habitat: Free-living. 14th June, 1956.

25. *Nitzschia longissima* (Bréb) Ralfs. Subrahmanyan, *op. cit.*, p. 191, Figs. 435-437; Van Heurck, *op. cit.*, p. 404, Pl. XVII, Fig. 568.

Cells solitary, central enlarged portion lanceolate, ends hair-like, elongated nearly straight (Text-fig. 6).

Breadth frustule, 5.7μ.
Length frutstule, 180.8—342μ.
Keel punctae, 12 in 10μ.
Habitat: Free-living. 14th June, 1956.



Text Figs. 20–30.—Fig. 20. *Surirella elegans* var. *tenuis* var nov. Fig. 21. *Gyrosigma balticum* (Ehr) Rabh. Fig. 22. *Pleurosigma galapagense* var. *kumariensis* var. nov. Fig. 23. *Nitzschia obtusa* var. *scalpelliformis* Grün. Fig. 24. *Bacillaria paradoxa* Gmelin. Fig. 25. *Pleurosigma spencerii* var. *curvata* Grün. Fig. 26. *Navicula cuspidata* f. *crassa* form nov. Fig. 27. *Pinnularia viridis* (Nitzsch) Ehr. Fig. 28. *Navicula cuspidata* var *ambigua* Venkataraman. Fig. 29. *Hantzschia amphioxis* var. *vivax* (Hantz) Grün. Fig. 30. *Amphora coffoeoformis* var. *africana* Fritsch and Rich. (Figs. 20–29, × 685; Fig. 30, × 1600).

26. *Nitzschia palea* (Kütz) W. Smith. Van Heurck, *op. cit.*, p. 401, Pl. 17, Fig. 554; Venkataraman, *op. cit.*, p. 353, Fig. 146.

Valves linear to linear-lanceolate, striations delicate (Text-fig. 7).
Breadth frustule, 3.8μ.
Length frustule, 30.4μ.
Habitat: Free-living. 17th June, 1956.

27. *Nitzschia tryblionella* Hantzsch. var. *levidensis* (Smith) Grün. Van Heurck, *op. cit.*, p. 385, Pl. 15, Fig. 494; Venkataraman, *op. cit.*, p. 352. Fig. 141.

Valves linear with slightly concave margins, ends wedge-shaped, striae clear (Text-fig. 16).
Breadth frustule, 7.6–9.5μ.
Length frustule, 68.4μ.
Striae, 11–14 in 10μ.
Habitat: Bottom living in saline pools. 17th June, 1956.

28. *Nitzschia obtusa* W. Smith. var. *scalpelliformis* Grün. Van Heurck, *op. cit.*, p. 397, Pl. 16, Fig. 538: Venkataraman, *op. cit.*, p. 255, Figs, 142, 147.

Frustules broad, keel fairly large. Two median keel punctae distant (Text-fig. 23).
Breadth frustule, 7.6—11.4μ.
Length frustule, 117.7μ.
Striae, 20–24 in 10μ.
This form is slightly narrower and longer than the type.
Habitat: Free-living. 15th June, 1956.

29. *Surirella elegans* var. *tenuis* var. nov.

Valves narrowly or broadly ovate, rounded at one end and acute at the other end, costae broad, wedge shaped in the girdle view (Text-fig. 20).
Breadth frustule, 49.4—57.4μ.
Length frustule, 110.2—144.4μ.
Habitat: Bottom living in saline pools. 16th June, 1956.
This form differs from the type in much smaller frustules.

ACKNOWLEDGEMENTS

In conclusion, the author wishes to express his great indebtedness to Dr. M. S. Randhawa for his keen interest and valuable criticisms throughout the course of this investigation. He is also grateful to Dr. B. P. Pal and Dr. S. M. Sikka for kindly providing the facilities to carry out this work.

Statement showing the distribution of the forms recorded in this paper in the Indian region.

	Previous places of collection	Authors
Amphora coffocoformis var. *africana* f. *kurze* form nov.	....	....
Bacillaria paradoxa Gmelin.	Madras coast	R. Gopala Iyer & Sankara Menon (1936).
	Madras	Venkataraman (1939).
	Madras	Subrahmanyan (1946).
	Porbandar	Misra (1956).
Cymbella turgida (Greg) Cleve.	Island of Banka	A. Grumon (1865).
	Madras	Venkataraman (1939).
	Darwar	Gandhi (1956).

Statement showing the distribution of the forms recorded in this paper in the Indian region.

	Previous places of collection	Authors
Cymbella cistula var. *maculata* (Kütz) Heurck.	Vaiyampatti	Venkataraman (1939).
Eunotia monodon Éhr.	Batang valley, Sikkim	Dickie, George (1882).
	Himalayas,	Skvortzow (1930).
	Peradeniya, Ceylon, Madras	Venkataraman (1939).
Gyrosigma balticum (Éhr) Rabbh.	Ceylon	Fortmoral.
	Madras	Venkataraman (1939), Subrahmanyan (1946).
	Bombay	Gonzalves & Gandhi (1954).
Gomphonema parvulum var. *subcapitata* Heurck.	....	....
Hantzschia amphioxis var. *vivax* (Hantz.) Grün.	Ceylon	Skvortzow (1930).
	Madras	Venkataraman (1939).
Melosira granulata var. *anguistissima* Müll.	Madras	Venkataraman (1939).
Navicula fusca var. *hyperborea* Heurck.	....	....
Navicula elliptica var. *ovalis* Hilse.	....	....
Navicula halophila f. *subcapitata* Östrup.	Madras	Venkataraman (1939).
Navicula hasta Pento.	Madras	Venkataraman (1939).
Navicula cuspidata var. *conspicua* f. *crassa* f. nov.	....	....
Navicula cuspidata var. *ambigua* Venk.	Madras	Venkataraman (1939).
	Bombay	Gonzalves & Gandhi (1954).
Nitzschia longissima (Bréb) Ralfs.	Madras	Subrahmanyan (1946).
Nitzschia palea (Kütz) Smith.	Vizagapatam	W. & G. S. West (1907), Skvortzow (1935).
	Ceylon	Skvortzow (1935) and W. & G. S. West (1901-5).
	Madras	Venkataraman (1939).
Nitzschia tryblionella var. *levidensis* (Smith) Grün.	Madras	Venkataraman (1939).
Nitzschia obtusa var. *scalpeliformis* Grün.	Calcutta	Skvortzow (1935).
	Madras	Venkataraman (1939).
	Darwar	Gandhi (1956).
Pinnularia viridis (Nitz) Éhr.	Burma	W. & G. S. West (1907).
	Madras	Venkataraman (1939).

Statement showing the distribution of the forms recorded in this paper in the Indian region

	Previous places of collection	Authors
Pinnularia interrupta f. *genuina* Fritsch.	Madras	Venkataraman (1939).
Pleurosigma spencerii var. *curvata* Grün	....	....
Pleurosigma galapagense var. *kumariensis* var. nov.	....	....
Rhopalodia gibba.	Manipur Madras	K. Biswas (1936). Venkataraman (1939).
Synedra ulna var. *oxyrhyncus* (Kütz) Heurck.	Madras	Venkataraman (1939).
Synedra ulna var. *crassa* var. nov.	....	....
Synedra ulna var. *amphirhyncus* (Ehr) Grün.	Madras, Trichinopoly, Nilgris Darwar	Venkataraman (1939). Gandhi (1956).
Surirella elegans var. *tenuis* var. nov.	....	....
Terpsinoe musica Ehr.	Ceylon Ceylon Madras	W. & G. S. West (1901-8). Skvortzow (1930). Venkataraman (1939).

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STUDIES ON THE SITE OF CONVERSION OF PTEROYL GLUTAMIC ACID TO CITROVORUM FACTOR IN RATS

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It was observed by Sauberlich (1949) that administration of pteroyl glutamic acid (PGA) in rats produced an increased urinary excretion of a factor required for the growth of the organism, *Leuconstoc citrovorum* 8061. The factor was termed *citrivorum factor* (CF) and was identified by Pohland *et al.* (1951) as a reduced and formylated derivative of PGA, N^5-formyl tetrahydro PGA. Guggenheim *et al.* (1956) observed that urinary excretions of PGA and CF increased considerably after feeding PGA to rats and the increase was further enhanaced by the addition of ascorbic acid to PGA. Black *et al.* (1941) observed that when rats were fed with a synthetic diet containing 0.5 per cent sulfaguanidine the animals grew at a very slow rate. Nielsen and Elvehjem (1942) reported that PGA counteracted the growth inhibition of sulfathiazole, and Dietrich *et al.* (1950) were of the opinion that PGA and vitamin B_{12} were synthesized by the intestinal micro-organisms which led to the increased accumulation of these vitamins in the liver. Ferrari (1955) reported that rats injected with carbon tetrachloride excreted less CF than normal rats after an intraperitoneal injection of PGA, although the total PGA activity of the urine showed no significant difference between the rats differently treated. Nichol and Welch (1950) observed that liver was the principal site of conversion of PGA to CF in the body and an enzyme system seems to be responsible for maximum conversion of PGA to CF (Doctor *et al.*, 1954).

The present communication deals with the studies carried out to ascertain the site of conversion of PGA to CF *in vivo* and *in vitro*.

EXPERIMENTAL

Male rats of weights varying between 150 and 200 gm. were used. The animals were fed with mineralised (Schantz *et al.*, 1938) whole milk with two drops of a concentrate of vitamin A and D, twice a week. The rats were housed in metabolism cages and 24-hour urine samples were collected in conical flasks under toluene for consecutive three days and urinary excretions of PGA and CF were estimated. The rats were then fed with 100γ PGA for consecutive three days by stomach tube and urine samples were collected every day. After a few days when the urinary excretions of PGA and CF reached normal levels, the rats were given 100γ PGA by intraperitoneal injections for consecutive three days and daily urine samples were collected. When the excretions of PGA and CF reached normal values, the animals were fed with 125 mg. sulfasuxidine for consecutive three days by stomach tube and then a supplement of 100γ PGA together with 125 mg. sulfasuxidine were introduced by stomach tube and the urinary samples collected as before. The animals were then fed with 31.25 mg. chloromycetin palmitate for three days followed by a supplement of 100γ PGA along with chloromycetin for another three days.

The rats were then given intraperitoneal injections of carbon tetrachloride (0.03 c.c./100 gm. body wt.) for ten days and when the animals showed fall in weight and aversion for food they were given intraperitoneal injections of 100γ

PGA for three days and the injections of carbon tetrachloride were continued, Urinary excretions of PGA and CF were estimated in the different urine samples throughout the experimental period. PGA was estimated by the differential microbiological assay method described by Weiland *et al.* (1952) using *Streptococcus faecalis* R. as the organism and CF was determined using *Leuconstoc citrovorum* 8081 by the method of Sauberlich and Baumann (1948).

The results are given in Table I.

TABLE I

Average 24 hours urinary excretions of pteroyl glutamic acid (PGA) and citrovorum factor (CF) by rats fed with different supplements

Supplement	PGA (γ)	CF (γ)
None (8)	1.04* ± 0.08	0.077 ± 0.003
PGA[1] (8)	10.40 ± 0.63	0.155 ± 0.023
Sulfasuxidine[2] (4)	0.49 ± 0.06	0.058 ± 0.007
Sulfasuxidine[2] (4) + PGA	14.73 ± 1.70	0.343 ± 0.041
Chloromycetin palmitate[3] (4)	0.49 ± 0.05	0.050 ± 0.004
Chloromycetin palmitate (4) + PGA	18.97 ± 1.13	0.326 ± 0.001
PGA[4] injection (8)	29.30 ± 4.05	0.413 ± 0.025
CCl_4[5] injection (7)	0.53 ± 0.05	0.042 ± 0.003
CCl_4[5] injection + PGA injection.	31.40 ± 2.54	0.416 ± 0.024
CCl_4 injection + PGA[1] (4)	9.40 ± 0.30	0.335 ± 0.03

[1] 100γ PGA fed per animal per day for 3 days.
[2] 125 mg. sulfasuxidine fed per animal per day for 3 days.
[3] 31.25 mg. chloromycetin palmitate fed per animal per day for 3 days.
[4] 100γ PGA injected intraperitoneally per animal per day for 3 days.
[5] 0.03 c.c./100 mg. body weight CCl_4 injected intraperitoneally per animal per day for 10 days.
Figures in parenthesis indicate the number of animals.
* Mean ± Standard Error.

At the end of the experiment the carbon tetrachloride treated rats and normal rats were killed after an over-night fast by decapitation. Liver. intestine, kidney, brain, spleen and pancreas were removed, chilled, adherent blood soaked and portions of the tissues were weighed. A 20 per cent homogenate of the different tissues were prepared with ice cold 0.08 M sodium phosphate of pH 6.3 and 5 c.c. of the homogenate were taken in 50 c.c. conical flasks. To each flask 5 c.c. of phosphate buffer of pH 6.3, 1 c.c. of a solution containing 100γ PGA and 1 c.c. toluene were added. The control flask contained 1 c.c. of carbon dioxide free water in place of PGA. Air in the flasks were replaced by a current of nitrogen, flasks stoppered, incubated for 4 hours at 37°C, stoppers replaced by cotton wool plugs, autoclaved for 5 minutes at 10 lbs. pressure, contents transferred to 100 c.c. volumetric flasks, neutralised to pH 6.8 with sodium hydroxide solution with bromothymol blue as external indicator, diluted to 100 c.c. with water and filtered. An aliquot of the filtrate was diluted suitably for the determination of PGA and CF by the microbiological assay method as used in these estimations in urine samples.

Percentage of PGA converted to CF and percentage of PGA destroyed, if any, by the different tissues were calculated and the results are presented in Table II.

TABLE II

Conversion of PGA to CF and destruction of PGA by the tissues of rats when 1 gm. tissue is incubated with 100γ PGA in phosphate buffer of pH 6.3 in an atmosphere of nitrogen for 4 hours (average of 7 observations).

Tissues	Conversion of PGA to CF (%)		Destruction of PGA (%)	
	Normal	CCl_4-treated	Normal	CCl_4-treated
Liver	4.82 ± 0.20	0.06 ± 0.01	17.4 ± 0.7	33.8 ± 4.4
Small intestine	0.03 ± 0	0.01 ± 0	6.9 ± 0.9	8.0 ± 1.1
Kidney	0.04 ± 0	0.04 ± 0	10.0 ± 1.0	12.9 ± 0.9
Brain	0.01 ± 0	0.01 ± 0	9.1 ± 1.1	10.7 ± 1.0
Spleen	0.03 ± 0	0.02 ± 0	10.3 ± 1.3	11.6 ± 1.3
Pancreas	0.02 ± 0	0.02 ± 0	1.7 ± 0.5	10.5 ± 0.9

DISCUSSION

Increased urinary excretions of PGA and CF were observed in rats fed with PGA. This was in confirmation of the works reported by other workers (Guggenheim *et al.* 1956). Comparatively less excretion of CF, however, was observed when PGA was injected intraperitoneally. This was possibly due to rapid rate of absorption of PGA and its distribution in the general circulation so that less time was taken for the conversion of PGA to CF by the tissues.

When the rats were fed with sulfasuxidine the urinary excretions of PGA and CF diminished. Intestinal bacteria synthesises PGA and sulfasuxidine retards their growth (Dietrich *et al.*, 1950). As less PGA is synthesised after sulfasuxidine less amount of it is converted into CF leading to its diminished excretion in urine. When PGA was fed to rats treated with sulfasuxidine there was increased excretion of PGA than the normal animals fed with PGA but the excretion of CF was lesser than the normal excretion. This indicates that possibly intestinal flora plays some rôle in the conversion of PGA to CF. Chloromycetin palmitate behaves in the similar way as sulfasuxidine thus emphasising the possible rôle of intestinal flora in the conversion of PGA to CF.

After injection of carbon tetrachloride the urinary excretion of PGA and CF greatly diminished. Carbon tetrachloride-treated rats excreted lesser amounts of both PGA and CF in urine after they were fed with PGA. Lesser excretion of PGA might be due to its destruction and diminished excretion of CF was possibly due to the diminished conversion of PGA to CF in the liver which is damaged by carbon tetrachloride. The intraperitoneal injection of PGA in carbon tetrachloride treated rats did not produce any apparent change in the urinary excretions of PGA and CF which was unlike the observation of Ferrari (*loc. cit.*). This might indicate that tissues of the body other than the liver are also concerned in the PGA metabolism in the body.

In vitro studies with different tissues of normal rats indicated that liver was mainly concerned in the conversion of PGA to CF. The other tissues studied,

namely small intestine, kidney, brain, spleen and pancreas played insignificant rôle in the conversion. When tissue homogenates were incubated with PGA it was not recovered completely which possibly indicated its destruction. The maximum destruction of PGA was observed with the liver and the destruction by other tissues was in the following order—kidney, spleen, brain, small intenstine and pancreas.

Carbon tetrachloride-treated rat tissues could convert PGA to CF less efficiently. The conversion of PGA to CF was very much depressed in the liver and small intestine. Destruction of PGA was also greater with the tissues of carbon tetrachloride treated rats. Although liver has been found to be the principal tissue concerned in the conversion of PGA to CF, destruction of PGA was also maximum with the liver tissue. Tissues might contain some enzyme which helps in the disintegration of PGA. Liver, small intestine and intestinal flora seem to be primarily concerned in the transformation of PGA to CF.

Summary

Daily urinary excretions of PGA and CF were determined in normal rats, in rats fed with sulfasuxidine and chloromycetin and in rats injected with carbon tetrachloride both before and after administration of PGA.

Urinary excretions of PGA and CF diminished when the rats were fed with sulfasuxidine and chloromycetin and also when they were treated with carbon tetrachloride.

Different tissues e.g., liver, small intestine, kidney, pancreas, spleen and brain from normal and carbon tetrachloride treated rats were incubated with PGA for 4 hours and PGA and CF were estimated.

More CF was formed by liver and small intestine from normal rats while the other tissues had practically no effect in this conversion, and this conversion was greatly affected in the liver and small intestine from carbon tetrachloride treated rats.

Different tissues of normal rats destroyed PGA and the destruction was maximum with the liver and least with the pancreas. Tissues from carbon tetrachloride treated rats destroyed PGA to a greater degree.

Liver, small intestine and intestinal flora seem to be concerned in the conversion of PGA to CF.

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SOILS OF WEST BENGAL

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Introduction

In the state of West Bengal the major practice of cultivation is paddy and it is so intensively cultivated that it is hardly possible to demarcate the lands for rice grown and not rice grown as, out of the total area of about 12.3 million acres under cultivation, paddy occupies almost 10.0 million acres. Throughout the year the practice of paddy cultivation can be seen in some places or other as there are various varieties which can be grown well under different soil climatic conditions. Yet the main growing season of paddy is from June to December when the ideal climatic condition for paddy cultivation under waterlogged condition prevails all over the state. Though the annual average rainfall over the entire state exceeds 40 inches, the required minimum for growing paddy crops as stated by de Geus (1954), there remain to be investigated the factors that account for different performances like varietal, manurial and cultural practices of paddy at different places. Since these in turn depend mostly on the soil factor, a knowledge of different kinds of soils growing paddy is essential for proper formulation of agronomy, fertiliser practice, irrigation requirements and such others. We have been surveying these soils for the last four years and a system of classification of soils has been evolved as shown below (Table I).

The different types of soil occurring in West Bengal have been described and discussed in the following pages and a tentative map indicating their distribution has been given (Text-fig. 1). In the genetical classification of soils of the State the genetic method adopted by the Soil Survey Staff (1951), United States Department of Agriculture, has been followed.

Laterite and Lateritic Soils

These soils occur in the districts of Birbhum, Burdwan, Bankura and Midnapur. The land is undulating and has many tiny rivulets. These rivulets are dry except during rains. Soil erosion varies from slight to very high. At places the honeycomb structured laterite beds have been exposed. Surrounding these are terraced paddy fields, extending down to the bed of the rivulets. The paddy fields are yellowish grey on top and red below.

The soils are acidic (*p*H 5.5 to 6.5), poor in calcium, organic matter, available phosphate and in bases. The topsoils are poor in iron due to leaching and accumulation takes place in deep subsoils.

These soils in general are responsive to manuring; phosphate and nitrogen increase the yield by about 2.5 and 4 maunds per acre respectively.

Buried Laterite (*Occurring at a depth of 10 ft. and below*)

The occurrence of the honeycomb laterite pan with laterite below could be met with in the places examined. Over this has been laid down an alluvium, by the floods of the Dwarekeswar, the Damodar and the Cossaye. This might have

TABLE I

SOILS

- Laterite & Lateritic soils
  - True Laterite
  - Buried Laterite
- Red Soil
- Alluvial Soils
- Coastal Soils
- Terai Soil
- Colluvial & Skeletal soils
  - Soils of Transition series
  - Bengal Gondwana soil
  - Bengal Gneissic soil

- Saline Soil
- Non-saline Alkali soil
- Saline Alkali soil
- Degraded Alkali soil

- Soils of Tista Family*
- Soils of Vindhya Family
  - Vindhya Sub-Family, viz. Rajmahal (Ra), Damodar (Da) & Cossaye (Co)
    - Ra, Da, Co. Riverine Soils
    - Ra, Da, Co. Flat Land Soils
    - Ra, Da, Co. High Land Soils
- Soils of Ganga Family
  - Ganga Riverine Soils
  - Ganga Flant Land Soils
  - Ganga Upland Soils
  - Ganga Lowland Soils

* Study of different characteristics and their classification is under investigation.

occurred in prehistoric ages, when the adjoining coal bearing Gondwana tracts may have been formed due to violent geological disturbances. Over the laterite pan lies a layer of heavier soil with occasional dolomite in it. Here concretions do not occur in a layer, but are scattered in the profile from 8 ft. to 10 ft., varying in length from 2 to 5 inches. The concretions and the silty clay accumulation seem to be a riverborne deposit. Over these (the burried laterite and the dolomite ridden heavy soil), stands the present profile of Damodar flat land. The surface layer appears to be younger in formation. This tract occurs in the districts of Birbhum, Bankura, Burdwan and Midnapore. Increase in yield of paddy with nitrogen is not significant. When phosphate is added along with it high responses are obtained, indicating a typical phosphate deficient tract

Red Soils

These soils occur in the districts of Birbhum, Burdwan, Bankura, Midnapore, Malda and in West Dinajpore. The soils are coloured Red or Brown having variable thickness, with or without occasional lime concretions in the profile and morrum or feldspar below. Pisolitic concretions containing sesquioxide at places increase in great numbers. At some places these have become numerous and have formed laterite like blocks. At these places the surface vegetation peculiarly enough has also changed from Sal to Palas. When this happens the area gives chessboard appearance of Red soil, Lateritic soils and Laterites, and they occur in such a close proximity that it has been shown separately in the map as Lateritic Red soil group. These soils are mildly acidic (pH 6.0 to 6.6), poor in calcium, organic matter, available phosphate and bases. Iron is poor on the top and increases with depth. Paddy grown in these soils responds well to nitrogenous and phosphatic manuring.

Alluvial Soils

The soils of the alluvial tract can be divided into two families depending upon the nature of the parent material, i.e., alluvial deposits from which they have been derived. The coined names given to the families are:

Ganga Alluvium:—All these soils, which have originated from the Gangetic alluvium have been given the family name "Ganga alluvium".

Vindhya Alluvium:—The family name "Vindhya alluvium" has been coined for the soil association which has been formed from the alluvium brought down by the rivers, originating from Rajmahal hills and Chottanagpur plateau, a physiographic continuity of the Vindhya ranges.

Vindhya Family

Rajmahal, Damodar, Cossaye Riverine

These associations having different soil types are characterised by profiles, having layers, without a regular sequence, immature, irregular layeration, with occasional bands of sand. Sands are coarse and are yellowish brown in colour. These soil associations occur in the districts of Murshidabad, Birbhum, Bankura, Burdwan, Hooghly and Midnapore.

The positions of these associations have been indicated in the accompanying map. These associations have an innundated sandy phase, which gets periodically submerged. These soils respond well to nitrogen but give poor response to phosphate except in one or two soil types in these regions. These soils are mostly neutral (pH 6.5 to 7.2) and have an average lime and base status.

Rajmahal, Damodar, Cossaye Flat Lands

These soil associations occupy almost a flat topography, away from the influence of floods from which the Vindhya Riverine lands suffer. Due to incomplete weathering the distinct soil horizons could not be formed. Yet in the soil profiles layers can be distinguishable, to some extent by their colour, moisture contents, root penetration, texture and other physical characteristics.

There is very slight illuviation of sesquioxides in the lower layers. Calcium, potassium and clay indicate that a process of weathering and leaching has started, as a result of which pH tends to become slighly acidic. There are occasional dolomite concretions of fairly big sizes occurring at random in the profile. The mode of distribution of these concretions indicate that these are flood borne deposits and have not been formed *in situ*. Brown iron concretions of irregular size occur in lower layers which do not effervesce with hydrogen peroxide indicating absence of manganese in them.

These soil associations occur in the districts of Murshidabad, Birbhum, Bankura, Burdwan, Hooghly and Midnapore. Their locations have been shown in the accompanying map.

These soils are mildy acidic (pH 5.8 to 6.8); calcium, iron and other bases are low on top and increase with depth. They respond well to the application of nitrogen, whereas phosphate gives response only in the clay types where addition of nitrogen does not give increased paddy yield.

Rajmahal, Damodar, Cossaye Highlands

These soil associations occur in the districts of Murshidabad, Birbhum, Burdwan Hooghly, Bankura and Midnapore. Their positions are indicated in the accompanying map. These are associations of different soil types, having mature profiles. Leaching of clay, sesquioxides, alkaline earths and alkali metals and their accumulation in the lower horizon are evident. Mottlings are present. Dolomite concretions occur, often in thick layers. These soils are mildly acidic, pH. 5.8 to 6.9, which increases with depth. Calcium and other bases are low on top but increase in the subsoils. Phosphate content is low. Leaching of iron from top soil and accumulation in subsoil have taken place.

Response to nitrogen is low and to phosphate lower still. The yield of paddy can hardly be increased by 3 mds. per acre by application of nitrogen.

Ganga Family of Soils

Ganga Riverine

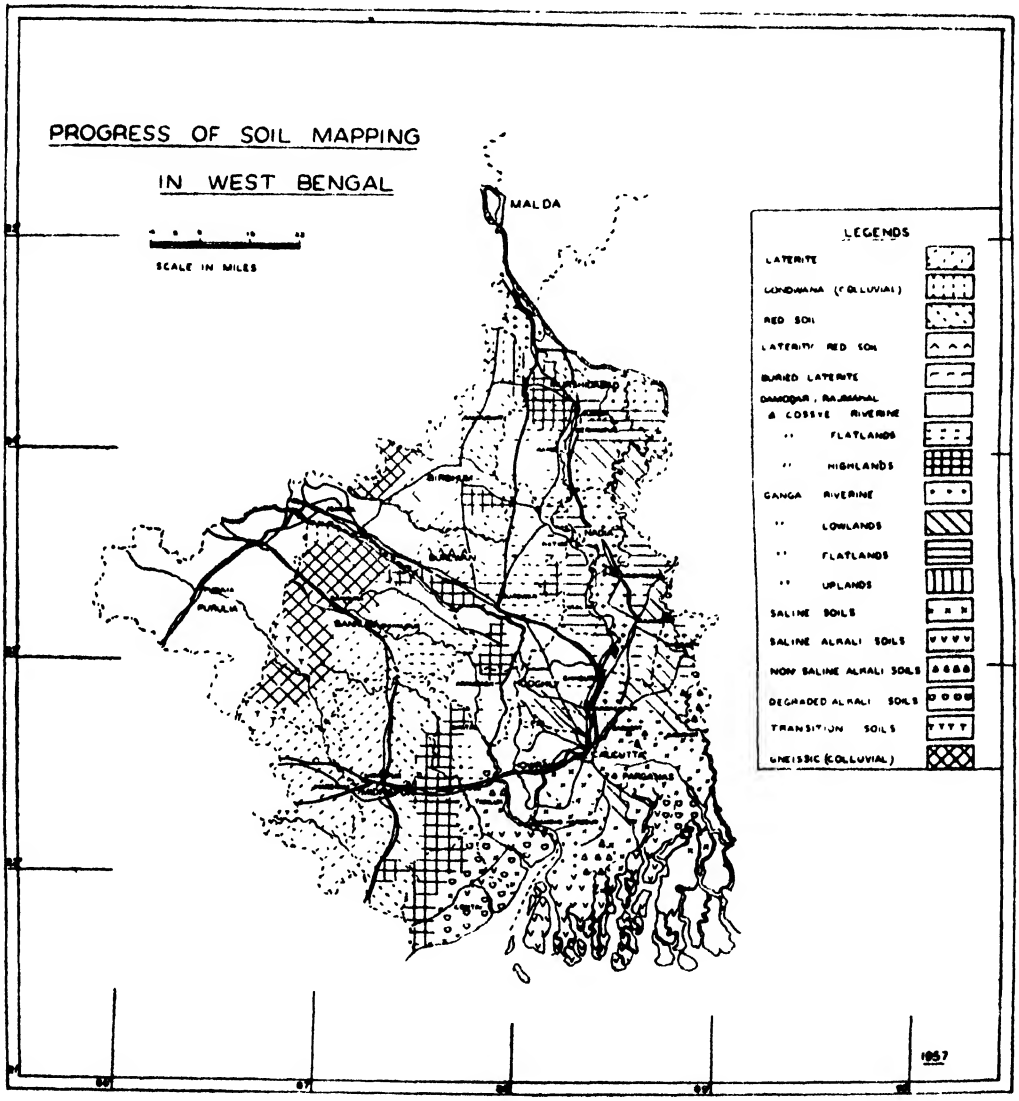
This kind of soil is found in the districts of Murshidabad, Malda, 24-Parganas' Burdwan and Hooghly and has been indicated on the map. This association of soils has an immature profile. Chemical and morphological observations show that one or two sandy layers are often found in the profiles examined. Sand fractions consist mostly of finer fractions, grey or greyish white in colour, having occasionally small plates of mica which shine in reflected light. These soils are rich in calcium. Free calcium carbonate occurs over most of the area either in the surface soils or within 2 to 3 ft. in the profile. The yield of paddy grain in this tract can hardly be increased by over 3 mds. per acre by application of nitrogen. Addition of phosphate does not give economical response.

Ganga Flat Land

This soil association occurs in the districts of Murshidabad, Nadia, 24-Parganas, Burdwan and Hooghly. It has a slightly more mature profile than the riverine soil. Slight illuviation of clay to the lower layer has taken place. Sesquioxides,

alkaline earths and metals all indicate slight leaching from the top and accumulation at the bottom. At places the subsoils still contain free calcium carbonate. The sand fraction is predominantly finer in size and whitish grey in colour.

No significant response in yield of paddy with phosphate fertilisers is found, whereas the increased yield of paddy with nitrogenous fertilisers is about 3 mds. per acre.



TEXT-FIG. 1.

Ganga Low Land

This association of soils occurs in places where depressions have been created due to the meandering of different rivers. The abandoned river courses and depressions, caused by strong diluviation by river currents, receive the washings from the surrounding flat or riverine lands. The Ganges also deposits a large amount of silt and clay in its diurnal tidal flows and ebbs. These factors have

been responsible for gradual filling up of river beds and marshes found in different localities. During rains these appear like lakes, where reeds mostly grow. Cultivation of *aman* paddy is carried out in the shallower places and of *boro* in the deeper ones.

This association is characterised by a profile having clay in the top horizon, followed by subsoils which are often very light, even consisting of riverborne loess or coarse sand. At places calcium carbonate concretions are met with. The profiles observed in this association of soils closely resemble lacustrine deposits, as stated by the U.S. Soil Survey Staff (1951), and have been found to lie over riverine profiles.

This occurs in the districts of Murshidabad, Nadia, 24-Parganas, and Hooghly. Their positions have been indicated on the map.

Field experiments show that application of nitrogen increases the yield of paddy by about 3 mds. per acre : the increase due to phosphate is not significant.

Ganga Upland

Soils on older alluvial fans, alluvial planes and terraces, have more or less well-developed profiles. These profiles have moderate accumulation of clay in them and a high concentration of lime in the form of concretions, in the subsoil, as a result of continuous movement of calcium from the surface horizons.

These soils are characterised by their clay like nature and the presence of a lime horizon. Sesquioxide and clay become immobile. The high clay content of the soil in the event of rising of level of water table below the ground to the surface during rainy season makes this type of land eminently suitable for cultivation of *aman* paddy. Phosphate contents of these soils are low and the same can be said for nitrogen and potash.

This soil association occurs in the districts of Murshidabad and Birbhum and its position is shown in the map.

Coastal Soils

These soils are of tidal origin. After alluvial tracts these form the next important *aman* paddy growing tract of the state. The soils of this tract have been formed from deposits brought by tidal currents. The original deltaic branches of the Ganges choked up because the headwater had been cut off, as a result of which numerous tidal flats were formed. These were subsequently bunded to prevent ingress of sea water. Sluices are constructed to allow the escape of rain water, which gradually dissolved away the salt from the soil. These soils are rich in plant nutrients and support a good stand of paddy, so long the rain water stands in the field to dilute the salt. Parent deposits are either rich in calcium or magnesium or consist of half decomposed organic matter.

Taking all these soil forming factors into account, soils are classified in the following types, as has been done by the U.S. Salinity Laboratory Staff (1954) after examining the soils in the field as well as analysing them in the laboratory.

(i) *Saline soils*, (ii) *Non-saline alkali soils*, (iii) *Saline alkali soils*, (iv) *Degraded alkali soils*.

These soils are met with in the districts of 24-Parganas, Midnapore and Howrah. Positions of these soil associations are shown in the accompanying map.

Saline soils

Soils with salt content greater than 0.15% and exchangeable sodium percentage less than 15, are grouped under this head. The pH of this kind of soil varies between neutral to slightly alkaline.

Saline Alkali soils

Soils with salt content greater than 0.15% and exchangeable sodium percentage more than 15, are grouped under this soil association. pH of this type of soil generally reads over 7.5 and the soil particles remain flocculated in a soil water mixture.

Non-saline Alkali soils

In this kind of soil the salt content is less than 0.15%, whereas the exchangeable sodium-percentage is greater than 15. pH of this kind of soil also lies on the alkaline side.

Degraded Alkali soils

Though these are Non-saline Alkali soils having exchangeable sodium percentage above 15, yet the pH is acidic. The lime content and to some extent the other base status of this kind of soil is low and acidic pH is for the presence of predominant hydrogen ion.

TERAI SOILS

The next group of soils which grow paddy are the Terai soils. They are derived from the mountain region of the Himalayas. These soils are brought down by the hilly rivers, the Tista, the Mahananda, the Torsa, the Jaldaka and their numerous tributaries which bring material from heights of above 10,000 ft. and deposit it about 200 to 300 ft. above sea level. The deposits are mostly sandy and of raw humus type and are deep black to grey black in colour. They occupy a good amount of paddy areas of Jalpaiguri, Darjeeling and Cooch-Behar districts.

These soils are very light in texture and are highly porous. During rains, due to the precipitation becoming greater than the rate of infiltration through these soils, the area gets water-logged as a result of which *aman* paddy can be grown.

Due to severe leaching by rain and presence of a good amount of organic matter the soils are acidic, pH 4.7 to 5.8, and are poor in bases and available plant nutrients.

A combination of nitrogen and phosphate has been found to increase the yield of paddy.

COLLUVIALS AND SKELETAL SOILS

The soils next in area are colluvials derived from the hills which are extension of the Chottanagpur plateau. These skeletal soils, containing large amounts of coarse sand and gravel grow poor and uncertain paddy crop and occur in the western part of Birbhum and Bankura and in the Asansol sub-division. These are divided into the following categories :

Bengal Gondwana Soils

These soils are derived from the parent rocks which have been formed during the Gondwana period.

Bengal Gneissic Soils

The soils that are formed from the parent Gneissic rocks.

Transition Soils

Parent rocks from which these soils are formed are of Sub-metamorphic type similar to those of Bijawar and Gwalior series.

TABLE II

Approximate Chemical Composition of the Soils (Constituents as per cent of air-dry soils)

Soils	*p*H	CaO	K_2O	P_2O_5	Carbon	Nitrogen
Laterite & Lateritic soils	5.5—6.5	0.1—0.4	0.1—0.4	0.01—0.05	0.05—0.5	0.01—0.08
Red Soils	6.0—6.6	0.1—0.5	0.1—0.8	0.01—0.05	0.05—0.5	0.01—0.05
Ganga Family:						
(a) Ganga-Riverine soils	7.5—8.2	1.0—5.0	0.3—0.7	0.10—0.15	0.20—0.3	0.02—0.05
(b) Ganga Flat land soils	7.0—8.0	1.0—6.5	0.4—1.0	0.10—0.15	0.30—0.5	0.04—0.06
(c) Ganga-Upland soils	6.0—7.5	0.5—2.0	0.1—0.4	0.03—0.06	0.30—0.5	0.03—0.06
(d) Ganga-Lowland soils	7.0—8.2	0.6—3.0	0.1—0.4	0.06—0.1	0.50—1.0	0.05—0.09
Vindhya Family:						
(a) Vindhya-Riverine soils	6.5—7.2	0.3—0.6	0.1—0.45	0.025-0.04	0.05—0.15	0.005-0.02
(b) Vindhya-Flat land soils	5.8—6.8	0.4—0.7	0.1—0.2	0.01—0.05	0.10—0.4	0.02—0.05
(c) Vindhya-High land soils	5.8—6.9	0.3—0.6	0.4—0.5	0.02—0.05	0.20—0.5	0.03—0.06
Coastal soils:						
(a) Non-Saline alkali soils	7.2—8.3	0.45-0.8	0.5—1.5	0.10—0.2	0.20—0.6	0.04—0.08
(b) Saline soils	6.5—7.6	0.6—0.8	0.3—1.0	0.06—0.1	0.30—0.8	0.05—0.1
(c) Degraded alkali soils	5.0—7.0	0.4—0.5	0.4—0.8	0.07—0.15	0.50—2.0	0.05—2.0
(d) Saline alkali soils	7.5—9.0	0.4—0.8	0.5—1.0	0.10—0.15	0.20—0.6	0.04—0.07
Terai soils	4.7—5.8	0.1—0.2	0.1—2.0	0.10—0.2	0.80—3.0	0.09—0.2
Colluvial & Skeletal Soils:						
(a) Gneissic	5.5—7.5	0.25-2.0	0.2—1.3	0.02—0.2	0.15—0.7	0.02—0.11
(b) Gondwana	6.0—7.0	0.10-0.50	0.2—0.6	0.02—0.06	0.30—0.8	0.03—0.08
(c) Transition	5.5—7.0	0.08-0.65	0.3—0.7	0.03—0.07	0.20—0.6	0.02—0.08

The areas where these types of soils occur have undulating topography. The paddy fields are terraced among the black coloured boulders of giant size or rock surfaces or hills or eroded lands. The land on the top of the undulated topography is often left without cultivation, due to the difficulty of holding rain water in those places. Those lands, when allowed to remain as such for long periods, turn gradually into Sal or Palas jungles.

Analytical Results

The chemical constituents that are required for the growth of paddy have been estimated in the above mentioned different types of soils following the method of analysis given by de Sigmond (1938), Williams (1928) and Wright (1939). The figures are shown in Table II.

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Summary

The soils of the State of West Bengal have been classified under different categories and their characteristics have been studied. The distribution of each kind of soil has been shown in an accompanying map. A brief description of each of these soils is given. Chemical constituents of these soils are also given.

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STUDIES ON THE PHYSIOLOGY OF RICE. XIII. DISTRIBUTION OF FREE AUXIN IN DIFFERENT ORGANS OF THE PLANT

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It has been reported previously from this laboratory (Sircar and Das 1954) that the endosperm of rice contains a large amount of auxin which gradually disappears with germination of the embryo. Further, Sircar, Das and Lahiri (1955) and Sircar and Lahiri (1957) have produced evidence of the presence of some factor in the endosperm which exerts a retarding effect on embryo growth in the initial stages of germination. From the results of a series of experiments in which fractions of endosperm were removed and endosperm extract or IAA substituted, they have presumed that this retarding factor in the endosperm is of auxin in nature which in supra-optimal concentration retards the growth rate of the embryo. The problem then arises wherefrom this auxin accumulates in the endosperm of rice, whether it is related with the development of the flower and what part of the flower contributes to its accumulation. The answer to these questions would obviously be of considerable importance to determine the auxin relations of rice plant.

Earlier workers, notably Muir (1942), Hatcher (1945), Lund (1956) and Nitsch (1950) have followed the changes and synthesis of auxin in tobacco, corn, strawberry and rye. Hatcher has shown that the auxin content of developing rye grain is very small upto 2 weeks after anthesis and during the next 3 weeks there is a rapid accumulation but as the grain ripens auxin disappears almost entirely. In order to locate the site of auxin formation Hatcher analysed the whole ear and concluded that in the ear of rye there are two systems of auxin production—one in the developing carpel and the other in the developing anther. In both these organs auxin first accumulates and then disappears. He also found that endosperm adjacent to the embryo is the region of auxin accumulation. The precise location of auxin in the endosperm was done by extracting separately the excised embryo, the aleurone and the endosperm. An increase in the auxin content of tobacco pistil after pollination has been noted by Muir (1942) and Lund (1956). With the background of these results the present investigation on the free auxin content of the developing stamens and carpels of rice and its distribution in the mature grain and early stages of germination was undertaken.

Materials and Methods

Method of auxin assay

Root inhibition test.—Root inhibition by the application of indolyl acetic acid bears a logarithmic relationship with the concentration of the solution used. This relation has been used by Moewus (1949) and Audus (1951) to develop a method for the assay of auxin in plants. Das (1953) made a detailed study of this method for the determination of auxin in rye using the same material for culture as test plant. By the root inhibition test slight variation of auxin can be detected. In this work the method devised by Das was adopted using rice root in place of rye as test plant. The details of the method are as follows : Rice grains (var. *Rupsail*) were soaked in distilled water for 24 hours at 25 ± 1°C in complete darkness. They were then sprouted on moist filter paper in petridishes for another 24 hours in

darkness at the same temperature conditions. After 24 hours, the germinating embryos just protruded from the husk. At this stage healthy seeds showing equal embryo development were selected out and transferred to sterilised agar slopes in test tubes containing auxin extract. Similarly, a set of control was prepared where no auxin extract was added. The test plants were allowed to grow for a period of 48 hours in dark chamber maintaining a temperature of $25 \pm 1°C$. At this stage rice seedling showed only primary root which was measured upto the correct mm. The mean root length was expressed as percentage of the control root growing on aqueous agar.

Computation of results. To determine quantitatively the amount of auxin present in a particular plant material, a calibration curve has been prepared. For this purpose IAA solutions ranging from 100mg/l to 10^{-5} mg/l in logarithmic concentrations were prepared and the percentage inhibition of root length produced by these ranges of concentration of IAA were determined. From this calibration curve the IAA equivalent in μg of a plant extract was determined.

Extraction of auxin.—Different methods for extraction of auxin from different plant materials have been tried by workers in this field. Went and Thimann (1937) found that whereas alcohol failed to extract auxin from oat grain, water-alcohol mixture gave intermediate values, varying with the proportion of alcohol used. Again Van Overbeek (1938) used water for extracting auxin from maize grain. In this laboratory Sircar and Das (1954) adopted water as solvent for free auxin from rice grain. The method proved to be a suitable one, accordingly in the present investigation auxin has been extracted with water. The fresh plant material was washed in tap water and then in distilled water to clear off external dirt. The weighed material was cut into pieces, small quantity of water added and then stored in complete darkness for 24 hours at 0°C in a refrigerator. Then the extract was filtered several times after washing with water. The filtrate was then diluted to 50 c.c. and an equal volume of 3 per cent agar was added. The agar extract was then plugged and autoclaved at 120°C for 15 minutes and were slanted at an angle of 85°. Control tubes containing only aqueous agar and tubes containing IAA media were also subjected to similar sterilisation. For each determination duplicate extracts were made and the auxin content of each extract was determined with 15-20 test plants.

The Change of Auxin Content in Different parts of Grain During Germination

Rice grains (var. *Rupsail*) were soaked in distilled water for 2, 4, 10, 14, 18 and 30 hours respectively. Soaked grains were then dissected under Zeiss Sterio Microscope into the following parts : (1) Embryo proper, (2) Scutellum, (3) Portion of the endosperm adhering to the scutellum, (4) Remaining upper half of the endosperm and (5) Husk.

The fresh weight of the tissues was determined accurately and their auxin content determined by the "Root inhibition test" method described previously. The concentration of auxin has been expressed in terms of the weight of air dry seeds, thus the error due to relative water absorption was eliminated. The results are prepresented graphically (Fig. 1) and in Table 1.

Results.—In air dried mature rice grain about $50\mu g$ of auxin is present per gram of endosperm of which the lower half of the endosperm contains slightly less than the upper half. The amount of auxin present in an embryo is $0.144\mu g$ of IAA Eq. while the scutellum contains comparatively negligible amount ($0.002\mu g$ of IAA Eq.). With soaking, hydration of the tissue begins, as a consequence of which changes in the concentration of auxin takes place in different parts of the grain (Fig. 1). After 2 hours of soaking a sudden rise in the auxin concen-

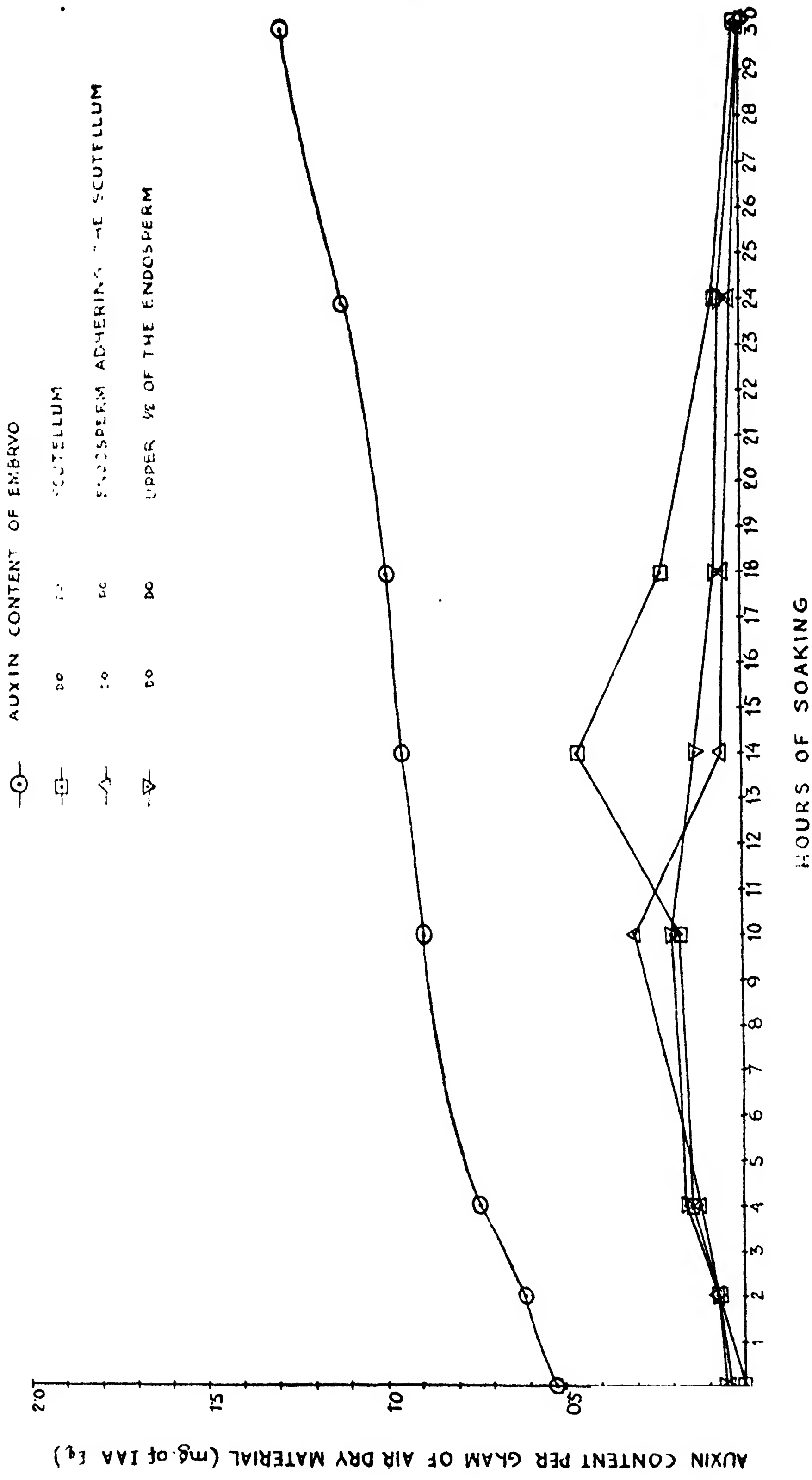
AUXIN CONTENT OF EMBRYO
DO DO SCUTELLUM
DO DO ENDOSPERM ADHERING THE SCUTELLUM
DO DO UPPER ½ OF THE ENDOSPERM
HOURS OF SOAKING
AUXIN CONTENT PER GRAM OF AIR DRY MATERIAL (mg. of IAA Eq)
2·0
1·5
1·0
0·5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

Fig. 1

TABLE 1

Changes of auxin content in the grain during germination (μg. IAA Eq.)

Treatments i.e., hrs. of soaking	Embryo		Scutellum		Endosperm adhering to the scutellum		Upper half of endosperm		Husk	
	Total auxin of 50 embryos	Auxin conc. per gm. of air dry material	Total auxin of 50 scutellums	Auxin conc. per gm. of air dry material	Total auxin of 50 endosperm halves	Auxin conc. per gm. of air dry material	Total auxin of 50 upper halves of endosperm	Auxin conc. per gm. of air dry material	Total auxin of 50 husks	Auxin conc. per gm. of air dry material
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Dry seed	7.23	527.51	0.10	7.12	12.59	41.96	18.20	60.65	0.50	2.03
2 hours	8.49	619.78	1.00	71.20	26.30	87.67	25.12	83.73	0.45	1.81
4 hours	10.20	745.14	1.99	143.16	35.48	118.27	50.12	161.64	0.40	1.61
10 hours	12.27	895.21	2.34	168.25	79.43	294.71	63.10	203.53	0.40	1.61
14 hours	13.15	953.39	6.29	459.44	19.95	66.58	42.66	137.60	0.40	1.61
18 hours	14.09	1028.61	3.15	226.96	17.78	59.27	31.62	102.09	0.31	1.28
24 hours	15.81	1154.12	1.12	80.09	12.88	42.94	28.84	96.63	0.31	1.28
30 hours	17.74	1297.11	0.16	11.38	6.31	21.03	19.95	64.38	0.31	1.28

tration of the scutellum was noticed which increased upto 10 times the quantity present in the scutellum of dry grain. Thus a change from 0.002μg to 0.020μg of IAA Eq. took place after first two hours soaking. It seems that hydration of the tissue activated auxin precursor which caused such a rapid increase. After the next two hours of soaking the auxin concentration doubled and a gradual rate of increase followed upto 14 hours soaking. At this stage 0.126μg of IAA Eq. is present in the scutellum of each grain or if expressed otherwise 459.44μg of IAA Eq. is present per gm. of the scutellum of air dried seed. While studying the changes in the auxin content of endosperm it is evident that at the same stage i.e., after 14 hours soaking decrease in the auxin content of endosperm begins, which indicates the beginning of the utilisation of endosperm auxin by the growing embryo. After 14 hours soaking scutellum auxin is found to decrease. This decrease continues upto 30 hours when only minute amount of auxin is left (0.003μg of IAA Eq. per grain). At this stage the embryos are well sprouted. Auxin content of the embryo gradually increases with the onset of soaking. Rate of increase is slow and follows a more or less straight path (Fig. 1). In the course of 30 hours soaking the auxin in the embryo has increased about two and half times. Endosperm auxin increased from the beginning of soaking to 10 hours. During this period inactive precursor is presumably activated. In between 10 to 14 hours utilisation of the endosperm auxin by the embryo begins, as a consequence of which endosperm auxin begins to decrease (Table 1). This decrease after a period of 10 hours soaking is more marked in the lower half of the endosperm than the upper half. Similarly after 18, 24 and 30 hours soaking decrease of auxin content is more pronounced in the lower half of the endosperm than in the upper part. Inference from this can be drawn that auxin of the endosperm adjacent to the scutellum is first utilised. A very small amount is present in the husk and no remarkable change in the content is noticed during sucessive stages of germination.

Distribution of Auxin in Different Parts of the Seedling

The seedlings were grown in sand culture in July-Sept., 1955 at the experimental garden of the Department of Botany, Calcutta University. The method of sand culture and nutrients applied were the same as in the previous work (Sircar and Sen, 1941).

Results. (Fig. 2 and Table 2).—In mature rice grain about 80 per cent total auxin is present in the endosperm. While the embryo contains the rest, the endosperm shows the presence of 0.81μg of IAA Eq., husks contain practically none. In the sprouted embryo the auxin level increases from 0.14μg. of IAA Eq., it decreases to 0.64μg in the endosperm. It should be noted that the amount of auxin decreased in the endosperm is much greater than that accumulated in the coleoptile. In the second day i.e., one day after sprouting, auxin level of the coleoptile decreases to 0.08μg., while the reduction to 0.63μg is noticed in the endosperm. It thus appears that the rate of decrease of the endosperm auxin is comparatively slow at this stage. Auxin content of root at this stage has been estimated to be 0.03μg IAA Eq., which is half the amount present in the coleoptile. On the third day, coleoptile auxin decreases to a level of 0.01μg of IAA Eq. ; by that time the endosperm looses about 0.13μg of IAA Eq. At the age of 5 days, 1st leaf is fully developed and 57 mm. in length. the 2nd leaf with 32.3 mm. length is unfolding and the root length is 56.6 mm. Distribution of auxin in different parts of the seedlings shows that auxin accumulates in the leaves with growth. Young leaf, however, contains higher auxin which decreases as the leaf approaches maturity. At the age of 20 days the endosperm is completely exhausted and no extractable auxin is detected. Auxin content of the coleoptile is the highest in the sprouted grain and decreases with age, while the crown shows an

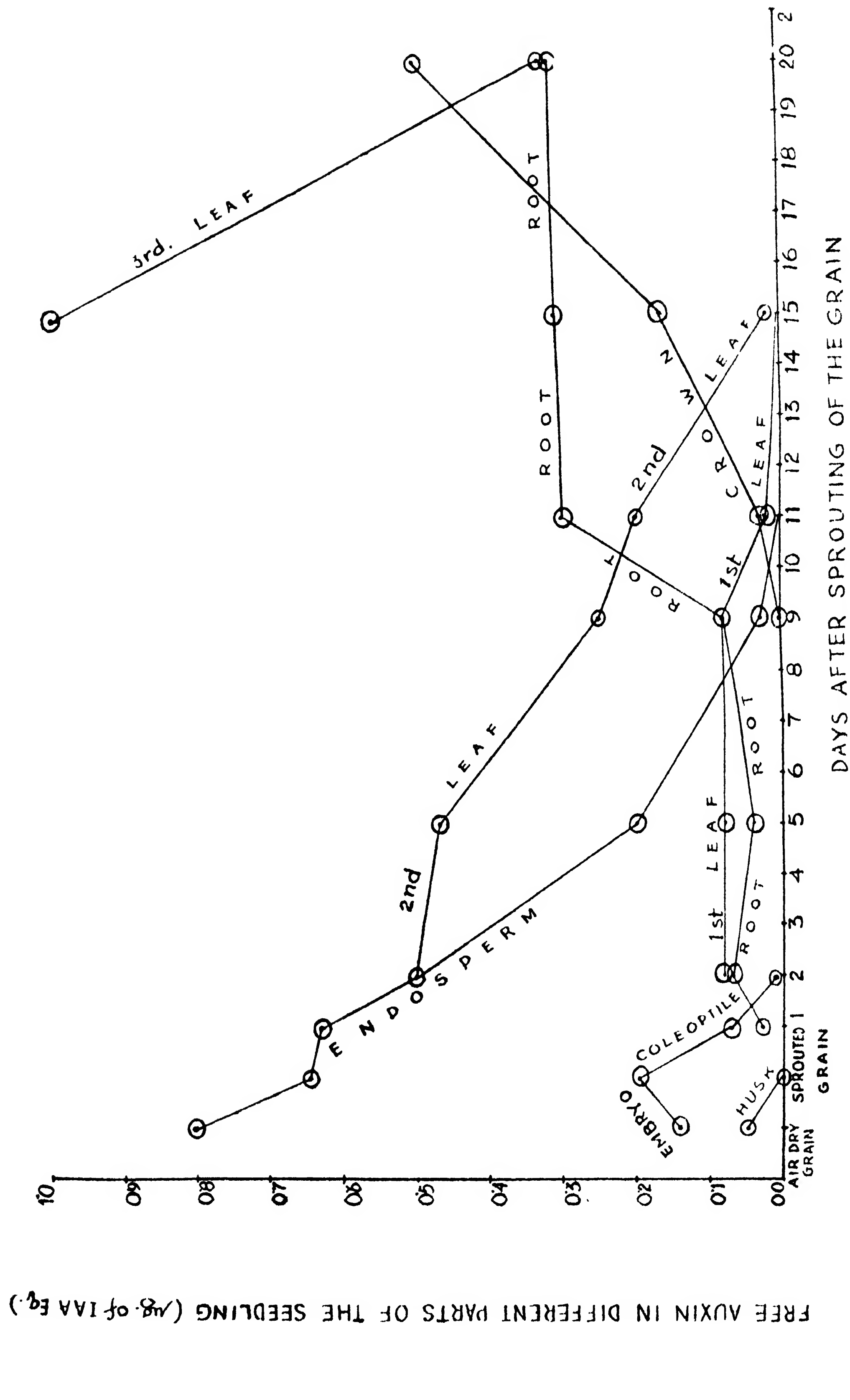
FREE AUXIN IN DIFFERENT PARTS OF THE SEEDLING (μg. of IAA Eq.)
DAYS AFTER SPROUTING OF THE GRAIN
AIR DRY GRAIN
SPROUTED GRAIN
0·0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
ENDOSPERM
EMBRYO
COLEOPTILE
HUSK
1st LEAF
2nd LEAF
3rd. LEAF
ROOT
CROWN ROOT

Fig. 2

TABLE 2

Auxin content of the different parts of the seedling

Age of the seedling	EMBRYO Fresh wt. of 10 embryos (gm)	EMBRYO Total free auxin per embryo µg. of IAA Eq.	EMBRYO Free auxin conc. on per gm. fresh wt. µg. of IAA Eq.	ENDOSPERM Fresh wt. of 10 embryos (gm)	ENDOSPERM Total free auxin per embryo µg. of IAA Eq.	ENDOSPERM Free auxin conc. per gm fresh wt. µg. of IAA Eq.	ROOT Fresh wt. of 10 embryos (gm)	ROOT Total free auxin per embryo µg. of IAA Eq.	ROOT Free auxin conc. per gm. fresh wt. µg. of IAA Eq.	CROWN Fresh wt. of 10 embryos (gm)	CROWN Total free auxin per embryo µg. of IAA Eq.	CROWN Free auxin conc. per gm. fresh wt. µg. of IAA Eq.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Air dry seed	0.0027	0.1445	527.4	0.119	0.8092	67.46	—	—				
Sprouted grain	0.03	0.199	66.35	0.2	0.6456	32.28	—					
Days after sprouting	COLEOPTILE											
1	0.01	0.07924	72.59	0.22	0.6308	28.67	0.015	0.0315	20.23			
2	0.0576	0.01101	1.74	0.222	0.5012	22.57	0.023	0.0706	30.71			
5				0.025	0.1995	9.24	0.149	0.0398	2.67			
9				0.197	0.0251	1.27	0.147	0.0794	5.40	0.12	0.0041	0.347
11				0.180	0.007	0.388	0.12	0.3311	25.9	0.12	0.0316	2.635
15							0.14	0.3162	22.58	0.20	0.1698	8.494
20							0.14	0.3235	23.82	0.21	0.5011	24.81

TABLE 2—contd.

Auxin content of the different parts of the seedling— contd.

Age of the seedling	FIRST LEAF			SECOND LEAF			THIRD LEAF		
	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo μg. of IAA Eq.	Free auxin conc. per gm. fresh wt. μg. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo μg. of IAA Eq.	Free auxin conc. per gm. fresh wt. μg. of IAA Eq.	Fresh wt. of 10 embryos (gm)	Total free auxin per embryo μg. of IAA Eq.	Free auxin conc. per gm. fresh wt. μg. of IAA Eq.
	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)
Days after sprouting									
1	—	—	—	—	—	—	—	—	—
2	0.202	0.0794	4.95	0.0739	0.5012	67.79	—	—	—
5	0.196	0.0793	4.046	0.099	0.4677	47.24	—	—	—
9	0.2	0.0794	3.971	0.24	0.2511	10.464	—	—	—
11	0.14	0.0255	1.763	0.225	0.1995	8.78	—	—	—
15	0.14	0.0035	0.253	0.229	0.0239	1.05	0.27	0.9953	36.53
20	—	—	—	—	—	—	0.28	0.3235	160.7

increase with the age of the plant. Some difficulty arises in interpreting the changes in the auxin content of the root. In root, growth usually takes place at the tip and so it is probable that growth-promoting substances are present in the tips. But during quantitative estimation the total amount of root present was considered. Hence an irregularity in the auxin content of root was noticed.

Auxin contents of developing stamens and carpels

Sampling.—The vegetative shoot apex of rice is very small and before floral initiation it remains completely encased within leaf primordia. Stem elongation, however, starts one to two weeks before floral initiation. Unlike rye and other temperate cereals it does not bear double ridges on the surface. The floral primordia first appear in the shape of globular protuberance on the sides of the slightly elongated shoot apex (Sircar and Sen 1953). Stamens are differentiated earlier than carpels. During emergence of flag leaf the inflorescence remains very young, about 6″ long. The husks are whitish and transparent. Stamens are only developed, their colour being greenish white ; carpels not yet formed. From a sample of 100 flowers, stamens and husks were carefully dissected with the help of a very fine sterilised needle. The stamens were then collected in a small tube, previously weighed with 1 c.c. water. The tube was kept surrounded with ice so as to prevent evaporation from the water surface during the time of collection. The husks were collected in another tube. The tubes were finally weighed and the net fresh weight of the material collected was thus obtained. The tubes were subsequently kept in a refrigerator. Next sampling was done 3 days after the flag leaf opened out ; carpels were formed at this stage and sampled separately.

Different stages at which the samples were made are as follows :—

Nov. 2, 1955	*1st stage*	Just after the emergence of the flag leaf, only stamens were developed; carpels not yet formed.
Nov. 5, 1955	*2nd stage*	Flag leaf opened out, but the spikelets were still within the flag. Carpels were formed.
Nov. 7, 1955	*3rd stage*	During the time of anthesis.
Nov. 14, 1955	*4th stage*	7 days after anthesis. From this stage only husks and carpels were collected as stamens withered out after anthesis.
Nov. 17, 1955	*5th stage*	10 days after anthesis ; milk stage.
Nov. 21, 1955	*6th stage*	14 days after anthesis, the endosperm became milky.
Nov. 25, 1955	*7th stage*	18 days after anthesis, the embryo was differentiated.
Nov. 28, 1955	*8th stage*	3 weeks after anthesis, the grain was mature.

Results. (Fig. 3 and Table 3).—When the flag leaves emerged the inflorescence enclosed within the flag leaf was soft, slender and transparent (3–5″ in length

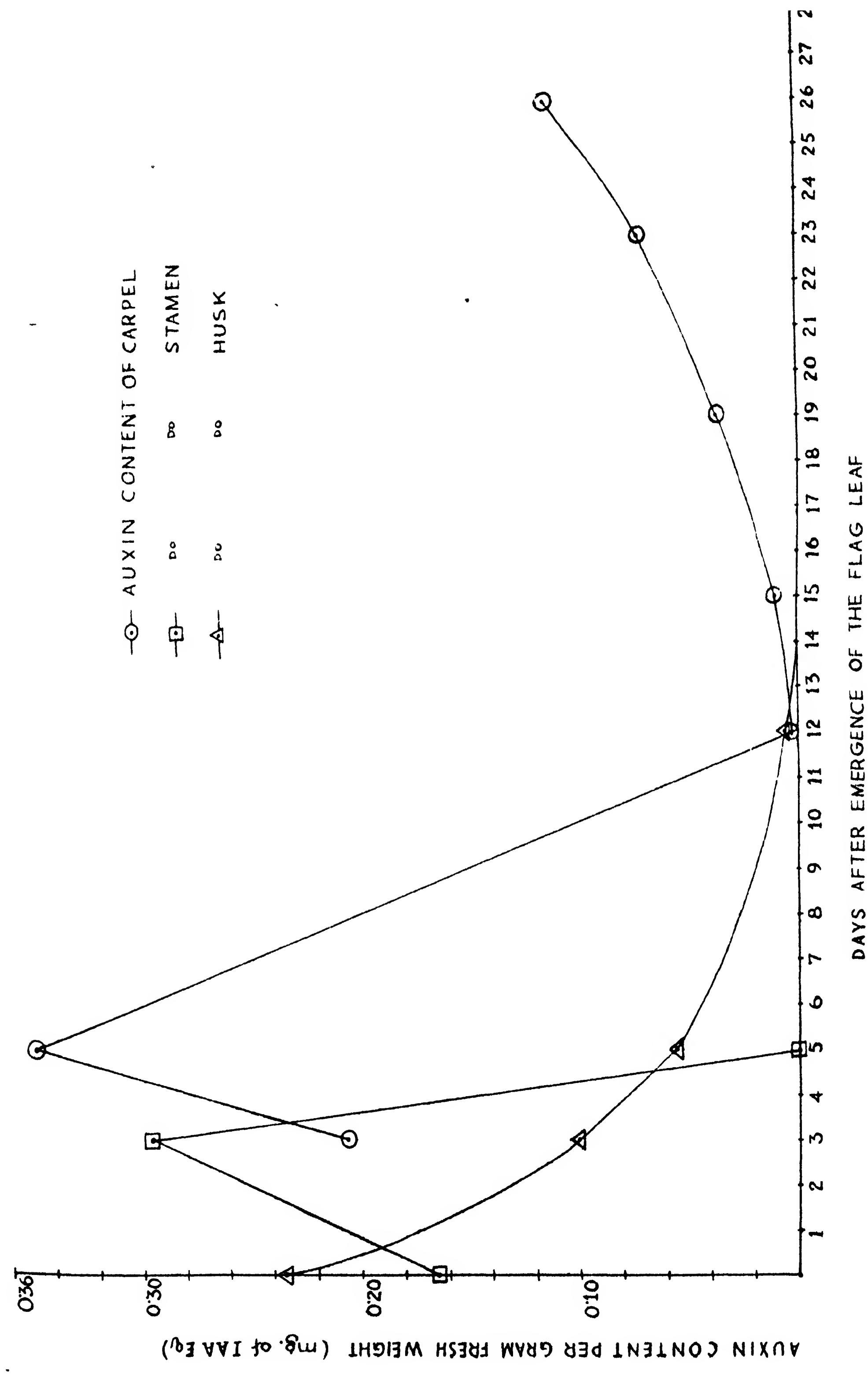
AUXIN CONTENT OF CARPEL
Do Do STAMEN
Do Do HUSK
AUXIN CONTENT PER GRAM FRESH WEIGHT (mg. of IAA Eq)
0·36
0·30
0·20
0·10
DAYS AFTER EMERGENCE OF THE FLAG LEAF
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 2

Fig. 3

TABLE 3

Auxin content of stamens, carpels and husk

Stages of development	CARPEL			STAMEN			HUSK		
	Fresh wt. of 100 carpels (gm.)	Total free auxin of 100 carpels μg of IAA Eq.	Free auxin conc. per gm. fresh wt. μg of IAA Eq.	Fresh wt. of 600 stamens gm.	Total free auxin of 600 stamens μg of IAA Eq.	Free auxin conc. per gm. fresh wt. μg of IAA Eq.	Fresh wt. of husks of 100 flowers gm.	Total free auxin of husks of 100 flowers μg of IAA Eq.	Free auxin conc. per gm. fresh wt. μg of IAA Eq.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
FIRST STAGE									
2nd. Nov. 1955— Just after the emergence of the flag leaf, only stamens are developed. Carpels have not yet formed.				0.024	3.972	165.0	0.15	35.39	235.98
5th Nov. 1955— Flag leaf has opened out, but the spikelets are still within flag leaf. Carpels are formed.	0.006	12.418	208.0	0.026	7.74	297.69	0.22	22.334	101.51
7th Nov. 1955— During the time of anthesis	0.014	49.097	350.67	0.026	0.012	0.482	0.2798	16.179	57.82
14th Nov. 1955— 7 days after anthesis	0.1208	0.35	2.95	—	—	—	0.2536	1.774	7.03
17th Nov. 1955— 10 days after anthesis	0.4331	5.012	11.56	—	—	—	0.2528	0.281	1.12
MILK STAGE									
21st Nov. 1955— Milk stage	0.6520	25.12	38.5	—	—	—	0.2520	0.002	0.007
25th Nov. 1955— Embryo differentiated at this stage	0.8022	58.88	73.42	—	—	—	0.2502	0.001	0.006
28th Nov. 1955— 3 weeks after anthesis. The grain is mature.	1.4264	162.18	113.63	—	—	—	0.3555	0.001	0.004

with small stamens 0.8 mm. in length). Three days after the unfolding of flag leaves, the stamens were still green and unripe ; but the size of the anthers and filaments increased and slight increase in the fresh weight was also noticed. As the inflorescence grew it became thicker and stouter and its green colour turned darker. On the 7th November, 1955 at about 10 A.M. the glumes opened at the mouth and stamens peeped through. This extrusion of the stamens signifies the time of anthesis. Six hours after anthesis the dehisced anther became yellowish and flaccid. Its size increased from 0.8 mm. to 2.4 mm. at the time of anthesis and increase was more rapid during the first half of its development. The rate of increase in fresh weight of the stamens was not parallel to the rate of increase in size. Even when the stamens were collected after anthesis their fresh weight remained the same as that found two days before. Carpels were first noticed on the 5th November, 1955 and were very small roundish, somewhat ovoid with feathery stigma. As the size of the carpel increased there was gradual increase in fresh weight. After anthesis the increase in the size of the carpel was noticeable. Seven days after anthesis the endosperm content was juicy and colourless and ten days after it became milky. This milk stage continued upto 14 days when the endosperm content became thicker. At about 2 weeks after anthesis the grain matured. Husks attained their full length, 7 days after anthesis but fresh weight was maximum at the anthesis (0.02798 gm./husk). After this the fresh weight of the husk gradually decreased though the size of the husk remained the same ; the husk gradually lost its water content and its colour became brownish after 17 days. But at an age of 3 weeks a slight increase in the fresh weight of the husks was noticed, which is possibly due to deposition of minerals. In rice (var. *Rupsail*) a large amount of auxin was present in unripe stamens i.e., at the stage when the stamens were green and unripe, largest amount (0.013 μg. of IAA Eq. per stamen) being present at the second stage when the flag leaf just unfolded, spikelets still enclosed and the carpels just formed. As the stamens turned yellow the auxin content gradually decreased several times while after anthesis very little auxin was detectable. Auxin level of very young carpels was very high (0.125 of IAA Eq. per carpel) and it increased 4 times after anthesis which took place 3 days after the full development of the carpels. In the stamens also, an increase in the auxin content was noticed 3 days after their full development. Thus increase of auxin content of carpels and stamens in the early stages of growth are parallel to each other. This is followed by a steep fall in the carpels after 7 days of anthesis. Auxin concentration again increased with the onset of milkstage which was practically the auxin content of the endosperm. It, however, did not attain the initial level of the carpel though the total auxin content of the endosperm was more than thrice that of the young carpel. Total auxin content of young embryo was much less than the total auxin content of the endosperm at the same stage. But auxin concentration of embryo and endosperm was more or less the same. Husks in their very early stages of development contained some amount of auxin which decreased regularly and at a stage of 7 days after anthesis there was practically nothing left. In the young husks growth and development continue, so it is probable that auxin present there is consumed with maturity.

Discussion

Change in auxin content in different parts of the grain during germination.—It appears that with soaking the auxin content of the embryo continues to increase. Obviously this comes from the endosperm. With different periods of soaking (upto 10 hours) diffusible quantity of auxin in the endosperm increases and upto 12 hours soaking it is also rising in the scutellum. Thereafter its presence in both scutellum

and endosperm decreases. Thus it appears that between 10-12 hours soaking endosperm auxin is translocated to the embryo through the scutellum. As the fall in the lower half of the endosperm is more sharp it is presumed that auxin of the endosperm next to the scutellum is first mobilised. With increasing periods of soaking presumably large amount is consumed by the sprouting embryo. It is very likely that this demand is met from the scutellum and the endosperm which have a decreasing level after 10-14 hours.

Distribution of auxin in different parts of the seedling in early stages of growth.—Diffusible amount of auxin has been estimated from endosperm, coleoptile, root and leaves of rice at different stages of germination. It has been shown that a large amount of auxin present in the endosperm gradually disappeared. Auxin level of the embryo was at first very low but with the sprouting of the grain, it increased rapidly and after reaching its peak it decreased again. Similar rise was noticed in the case of leaves and roots. Auxin is known to be present in the endosperm in large quantities as shown by Avery *et al.* (1940, 1941) in wheat, oat and maize ; Hatcher (1945) in rye and Das (1953) in rye. Evidence for the movement of auxin precursor from the base to the tip of coleoptile has been presented by Skoog (1937) and a gradual disappearance from the grain during germination has been shown by Sircar and Das (1954). In the present work the gradual loss of auxin released from the endosperm is not equal to the amount gained by the embryo during growth. Das (1953) has shown that without any participation of the endosperm some amount of auxin may be synthesised independently in the coleoptile during growth. This possibility together with that of Funke and Soding's (1948) observation that during transport auxin of the endosperm becomes inactive in the coleoptilar node and activated again in the coleoptile tip, may explain the discrepancy with the change of auxin balance in the embryo and endosperm ; as the amount of activated auxin may not be equal to the amount released from the endosperm. The results of the auxin relations of the growing embryo suggest that auxin is closely associated with its growth. The maximal rate of its production has been found to occur in the embryo just prior to the period of maximal growth rate i.e., the rate of auxin production follows a course parallel to the growth rate. It should be borne in mind that the amount which is available in the tissue at any instant is the balance between the amount of production and consumption during growth. It is possible that at the initial stage of growth the rate of production is much higher than that of the consumption, hence large accumulation resulted which helped the growth rate to reach its peak but after this the production probably stopped and the level of accumulated auxin gradually decreased by consumption. This possibility would go far to explain the fact that a large accumulation occurs prior to the grand period of growth and that the rate of auxin production follows a curve parallel to the growth rate.

Auxin content of developing stamens and carpels.—Wittwer (1943) found that an appreciable amount of auxin accumulated in the carpel of Zea after anthesis and he emphasised the phenomenon of reduction division as a cause of such accumulation. In this work a detailed study has been made on the relation of auxin to the development of different parts of the rice flower. Auxin was found to accumulate in the developing carpel upto the stage of anthesis. Such an accumulation possibly helps the growth of the carpel. After anthesis within 4-7 days there was a rapid fall in the concentration ; over 90 per cent was found to disappear from carpellary tissue at that period. Such a reduction was not observed by Wittwer. But Hatcher (1945) showed that the auxin content of winter rye rapidly increased after fertilisation and subsequently fell during the ripening of the grain. Das (1953) found that the auxin is gradually accumulated in the ear of vernalized or unvernalized winter rye under long day condition and decreased after the grand period of ear elongation. Again after the ear emergence concentration rises up. In rice such a rise of auxin concentration has been noticed after initial rapid fall. This

rise is presumably due to the accumulation of auxin in the developing endosperm tissues. Auxin content of the stamens of rice also presents an interesting picture. It gradually increased in the stamens and reached the peak during anthesis and then rapidly disappeared with withering of the tissue which indicates that it is closely associated with the growth of the stamen. Similar phenomenon was also observed in the case of the developing husk in which concentration of auxin was maximum at the beginning of growth and at maturity it almost disappeared.

Acknowledgement

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Summary

Distribution of auxin in different parts of rice grain during germination and its production in the stamens and carpels have been investigated using the root inhibition test. The test materials in these experiments were also the primary roots of rice seedling. The auxin content of the embryo increases gradually until sprouting. On the other hand the auxin content of the scutellum and the endosperm (both lower and upper half) rises to its peak value in between 10-14 hours and then a fall is noticed. In the sprouted grain amount of auxin in the embryo increases while a decrease in the endosperm is noticeable. But the amount decreased in the endosperm is greater than that accumulated in the coleoptile. Coleoptile auxin is highest in the sprouted grain and decreased with age. Young leaves show a high auxin content which is destroyed or inactivated at maturity. An increase in the amount of auxin along with the age of the plant is noticed in the crown. Root shows an increase which is maintained more or less constant. Auxin content of stamens gradually reaches a peak during anthesis which rapidly disappears with the withering of the tissues. Auxin level of carpels is also high, which increases about 4 times during the time of anthesis. After anthesis it rapidly decreases which again shows an increase with the onset of milk stage. Husks in their early stages of growth contain some amount of auxin which decreases at maturity. Total auxin content per young embryo is much less than the total auxin content of the endosperm but their auxin concentration is more or less the same.

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PRODUCTION OF ULTRAVIOLET INDUCED MUTATIONS IN *FUSARIUM VASINFECTUM* WITH SPECIAL REFERENCE TO FUSARIC ACID SYNTHESIS

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Introduction

In recent years many investigators have shown that ultraviolet and x-ray induced fungal mutants, in culture, may differ considerably from the parent in many physiological characters while being identical in gross morphology (Beadle and Tatum, 1945 ; Bonner, 1946 ; Raper *et al.*, 1945 ; Lockwood *et al.*, 1945). Mutation is known to occur spontaneously in Fusaria with either the loss in ability to produce mycelial growth in culture (Miller, 1946 ; Cormack, 1951 ; Venkata Ram, 1955) or in pathogenicity (Miller, 1945). In *Fusarium* infected plants the fungal toxin is known to play a vital part in the disease syndrome (Gäumann, 1951), but very little is known about toxin production in mutant strains, either naturally occurring or induced artificially, of plant pathogenic Fusaria. This paper presents the results of an investigation on the production of fusaric acid, one of the wilt toxins, *in vitro* in ultraviolet irradiated mutants of *F. vasinfectum*, the causal pathogen of the cotton wilt disease.

Materials and Methods

A monoconidial culture of *Fusarium vasinfectum* Atk., obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, was employed in this investigation. This isolate had retained its normal characteristics through ten successive cultural generations upon potato-dextrose agar medium and was therefore quite stable in culture. A shallow layer of a spore suspension in distilled water was taken from a ten-day old culture of the fungus in Petri dishes and irradiated at a distance of 25 cm. from the ultraviolet lamp for 1, 2, 3, 5, 10 and 20 minutes, control (unirradiated) being maintained. Two sources of ultraviolet radiation were used : (1) A 'Philips' 125W. lamp (type HPW) with a dome reflector which had peak radiation at 3655 A.U. This lamp was a super-high pressure mercury lamp consisting of a small discharge tube of quartz enveloped in an outer bulb of (black) Wood's glass and which according to the manufacturer's claim eliminates the use of separate filter. More than 95 per cent of the output was at wavelength 3655 A.U., about 2% radiation at 3350 A.U., 1% at 3125 A.U. and a total of 2% at 3900 A.U. and 4050 A.U. (2) A low-pressure high-voltage mercury vapour discharge lamp made by The Thermal Syndicate Ltd., (No. T/M5/369E) fitted with a metal reflector which gave most of the radiation at wavelength 2536 A.U. This type of high-voltage discharge in mercury vapour operating in an evacuated quartz tube generates almost entirely monochromatic radiation of wavelength 2536 A.U. The intensity of radiation, more than 99 per cent of which was of wavelength 2536 A.U. is specified by the makers as 121 micro-Watts per square cm. at 100 cm. from the lamp. The quantum of energy radiated by the lamp at the surface of a single Petri dish at a distance of 25 cm. from the lamp, per unit time, was calculated by chemical actinometry (Bowen, 1946). After irradiation,

aliquots of the spore suspension were added to melted potato-dextrose agar medium in 10 replicates, plated in Petri dishes and incubated at 25–29°C. Individual colonies developing from the spores were picked out in each treatment, including both obvious cultural and morphological variants and apparently normal appearing colonies, and transferred to potato-dextrose agar slants.

Pathogenicity of the irradiated and unirradiated strains was investigated on susceptible cotton (Karunganni 2—*Gossypium arboreum*) grown in sand culture in pots (16 seeds per pot in six replicates) by inoculating a spore and mycelial suspension of the fungus and determining the percentage of pre- and post-emergence infection at the end of 6 weeks' incubation in the glasshouse at 25–30°C. Fusaric acid production *in vitro* was studied by growing the strains on 50 ml. Richards's medium in 250 ml. conical flasks at 25–29°C for three weeks and testing the filtrate for fusaric acid by bioassay (Kalyanasundaram, 1955). The filtrate was concentrated *in vacuo* and the identity of fusaric acid determined by chromatography (Kalyanasundaram and Venkata Ram, 1956).

RESULTS

Details of investigations on unirradiated spores and those irradiated with ultraviolet light of wavelength 3655 A.U. are being omitted here since such spores did not produce colonies with any marked morphologically or physiologically altered characters; all such spores produced apparently normal colonies of the parent type (T-1, Table II). When 30 such cultures each from irradiated and unirradiated spores were tested, their pathogenicity and fusaric acid production *in vitro* was similar to that in the parent culture. Irradiation of *F. vasinfectum* spores with ultraviolet light of wavelength 3655 A.U. at a distance of 25 cm. up to 20 minutes was, therefore, ineffective in producing mutation.

Wavelength 2536 A.U. was lethal to *F. vasinfectum* and irradiation for periods over 10 minutes completely inactivated the spores. The number of colonies appearing from spores irradiated for longer periods decreased sharply (Table I). only 8 colonies developed from spores irradiated for 10 minutes in comparison to 307 when irradiated for 1 minute.

TABLE I

Showing the number of F. vasinfectum *colonies developing when irradiated with ultraviolet light of wavelength 2536 Å*

Treatment	Period of irradiation	Quantum of energy radiated in ergs	Total number of colonies
R-1	1 minute	2.473×10^7	307
R-2	2 minutes	4.946×10^7	172
R-3	3 ,,	7.419×10^7	90
R-5	5 ,,	12.365×10^7	46
R-10	10 ,,	24.730×10^7	8

From the colonies developing from the irradiated spores which had received different quanta of energy of wavelength 2536 A.U. (Table I), 30 isolates were picked and transferred on potato-dextrose agar slants. These 30 isolates, including 7

normal appearing strains and 23 cultural mutants (Table II), were found to be stable in culture through ten successive cultural generations upon potato-dextrose medium, and these were studied for their pathogenicity and fusaric acid production *in vitro*. In all, six culturally distinguishable mutant types were recognised (Table II) which differed from the parent in the characteristics of their aerial mycelium and stroma.

TABLE II

Cultural characters of the ultraviolet irradiated mutants of F. vasinfectum *studied for fusaric acid production*

Mutant types	Cultural characters	Total No. of strains	Strain Nos.
T-1	Normal; aerial mycelium moderate, felt like, smooth, pale violet, stroma dark violet, smooth, plectonchymatic.	7	v-1-2 v-1-5 v-1-7 v-1-10 v-1-13 v-5-27 v-10-29
T-2	Aerial mycelium abundant, pale violet, smooth, stroma violet, plectonchymatic, smooth.	9	v-1-3, v-1-8 v-2-14, v-2-16 v-3-19, v-3-20 v-5-24, v-5-28 v-10-30
T-3	Aerial mycelium abundant, white, smooth, stroma pale violet, plectenchymatic, smooth.	1	v-3-23
T-4	Aerial mycelium sparse, pink coloured, smooth, stroma carmine pink, smooth, plectenchymatic.	1	v-1-12
T-5	Aerial mycelium white, rough, stroma violet coloured, plectonchymatic.	3	v-1-1 v-1-11 v-3-21
T-6	Aerial mycelium abundant, rough, pale pink, stroma violet, plectonchymatic.	4	v-1-6 v-1-9 v-2-17 v-5-26
T-7	Aerial mycelium felt like, pale pink, smooth, stroma light violet, plectonchymatic.	5	v-1-4, v-2-15 v-2-18, v-3-22 v-5-25

Results of the pathogenicity tests and fusaric acid production are presented in Table III. Out of the 30 irradiated cultures, only 13 were capable of synthesizing fusaric acid *in vitro* and 10 of these produced more fusaric acid than the parent culture. Strains v-1-6 and v-5-25, both cultural mutants, produced highest quantities of fusaric acid, being six times more than that synthesized by the parent culture. In strains v-1-1, v-1-5, v-1-7 and v-1-13, fusaric acid detected was more than twice the quantity produced by the parent culture. Strains v-1-5, v-1-7 and v-1-13 were culturally identical to the parent isolate, whereas

v-1-1 was a cultural mutant (Table II). In total, 17 irradiated strains had lost their ability to synthesize the toxin in culture which represented a loss of more than 55 per cent in ability to produce fusaric acid due to ultraviolet (2536 A.U.) irradiation ; out of these 17 strains, 15 were cultural mutants whilst two, v-1-10 and v-5-27, were culturally identical to the parent type.

From Table III it is obvious that out of the 30 cultures studied, which were obtained from ultraviolet (2536 A.U.) irradiated spores of *F. vasinfectum*, only one

TABLE III

Fusaric acid production in vitro *and pathogenicity of the mutant strains of* F. vasinfectum *on susceptible cotton*

Strain	*Mutant type	Pathogenicity		Fusaric acid production in mg./l.
		Percentage pre-emergence	Percentage Post-emergence	
v-1-2	T-1 (parent type)	10	86	35
v-1-5	,,	24	100	120
v-1-7	,,	16	92	75
v-1-10	,,	16	60	nil
v-1-13	,,	12	98	75
v-5-27	,,	20	40	nil
v-10-29	,,	10	88	50
v-1-3	T-2	20	80	nil
v-1-8	,,	16	68	nil
v-2-14	,,	14	56	nil
v-2-16	,,	28	60	nil
v-3-19	,,	20	42	nil
v-3-20	,,	10	92	35
v-5-24	,,	10	90	nil
v-5-28	,,	14	62	nil
v-10-30	,,	18	96	50
v-3-23	T-3	8	38	nil
v-1-12	T-4	20	96	50
v-1-1	T-5	28	100	120
v-1-11	,,	10	80	nil
v-3-21	,,	16	88	35
v-1-6	T-6	26	100	220
v-1-9	,,	6	36	nil
v-2-17	,,	20	88	nil
v-5-26	,,	24	44	nil
v-1-4	T-7	12	64	nil
v-2-15	,,	26	52	nil
v-2-18	,,	10	68	nil
v-3-22	,,	28	86	43
v-5-25	,,	18	100	220
F. vasinfectum	(parent)	18	86	35

* Details of cultural characters are given in Table II.

(v-1-2) was not a mutant, being identical to the parent culture in both cultural and physiological characters, whereas the other 29 strains represented true mutation. Six cultures, v-1-5, v-1-7, v-1-10, v-1-13, v-5-27 and v-10-29, which were

unaltered in their cultural characters (normal appearing T-1 type), were found to have altered considerably in their ability to synthesize fusaric acid *in vitro* (Table III): these six cultures, therefore, represented physiological mutants. In the other 23 cultural mutants, the ability to synthesize fusaric acid remained unaltered in two (v-3-20 and v-3-21), increased in six cultures (v-1-1, v-1-6, v-1-12, v-3-22, v-5-25 and v-10-30) and was lost in the other cultures.

The cultural and physiological variations manifested by the ultraviolet irradiated spores represented true mutation and was not due to heterocaryosis because when 30 monoconidial cultures, obtained from the parent isolate, were similarly tested, they did not exhibit any variation in cultural or physiological characters.

With regard to the pathogenicity of the 30 cultures, obtained from ultraviolet (2536 A.U.) irradiated spores, with the exception of 5 cultures, v-1-9, v-3-19, v-3-23, v-5-26 and v-5-27, all the others produced more than 50 per cent post-emergence infection (Table III). None of the cultures had completely lost their pathogenicity, although fifteen of these were incapable of fusaric acid synthesis *in vitro*.

Discussion

The production of morphologically and biochemically altered mutants by ultraviolet irradiation has been reported in the case of *Aspergillus terreus* (Hollaender *et al.*, 1945; Raper *et al.*, 1945, and Lockwood *et al.*, 1945). Ultraviolet induced mutants which had strikingly altered nutritional requirements have been isolated in *Penicillium notatum-chrysogenum* (Bonner, 1946) and *Neurospora crassa* (Beadle and Tatum, 1945). Increased production of penicillin in mutants of *P. notatum-chrysogenum* (Gattani, 1952) and of itaconic acid in *A. terreus* (Raper *et al.*, 1945) have been obtained. In the present investigation, irradiation of *F. vasinfectum* spores with ultraviolet light having a wavelength of 2536 A.U. resulted in the production of certain mutants which had ability to synthesize fusaric acid more than six times the quantity produced by the parent culture under identical conditions (Table III).

Irradiation with wavelength of 3655 A.U. up to a period of 20 minutes was ineffective in producing mutations but radiation with 2536 A.U. even for one minute produced morphologically and biochemically altered mutants. Exposure to wavelength of 2536 A.U. when the quantum of energy received exceeded 24.73×10^7 ergs resulted in the complete inactivation of all the spores (Table I). Six culturally different mutant types were produced by the irradiated spores (Table II) but fusaric acid synthesis in the mutant strains was not related to their cultural characteristics (Table III). Strains which synthesized fusaric acid as well as those which had lost their ability to produce the toxin in culture were found to have identical cultural characters. Although the strains incapable of synthesizing fusaric acid generally caused less infection in cotton than those which produced the toxin in culture (Table III), fusaric acid synthesis *in vitro* did not seem to be related to the pathogenicity of the isolate. These results are in agreement with the findings of Gäumann *et al.* (1950), who demonstrated that the avirulent strains of *F. lycopersici* produced more of the phytotoxin lycomarasmin in culture than the virulent strain. Kern (1952) further showed that both lycomarasmin and fusaric acid are produced in greater quantities by the avirulent strain than the virulent ones.

Ten strains, which were obtained from ultraviolet (2536 A.U.) irradiated spores, synthesized greater quantities of fusaric acid in culture than the parent isolate and of these only six strains had altered cultural characters; the majority of the culturally changed mutants had lost the ability to produce fusaric acid (Table III). The production of ultraviolet induced mutation appears to offer somewhat limited possibilities of increasing yields of fusaric acid from *F. vasinfectum*.

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Summary

Irradiation of spores of selected strain of *Fusarium vasinfectum* with ultraviolet light (wavelength 2536 A.U.) resulted in the production of mutants with altered morphological and physiological characteristics. Exposure to radiation of energy exceeding 24.73×10^7 ergs at this wavelengths killed all the spores. Out of a total of 30 irradiated strains investigated, which had remained stable in culture through ten successive cultural generations on potato-dextrose agar media, 10 were capable of synthesizing greater quantities of fusaric acid *in vitro* than the parent culture and of these only 6 appeared as cultural mutants. The majority of the culturally altered strains had lost their ability to produce fusaric acid. The variation in cultural and physiological characters manifested by the strains obtained from ultraviolet irradiated spores represented true mutation and was not attributable to heterocaryosis. Increase in production of fusaric acid in culture does not seem to be a preferred reaction of ultraviolet induced mutation in *F. vasinfectum* because only about 33 per cent of the mutants were capable of increased fusaric acid production than the original parent isolate.

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EFFECT OF 19-NORTESTOSTERONE ON THE GENITAL ORGANS AND PREGNANCY IN RATS

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A. INTRODUCTION

The possibility of physiologic control of fertility by the use of sex hormones or their chemical relatives for family planning and reduction of world's population pressure is currently considered with great interest (see Henshaw, 1955; Nelson, 1955; Parkes, 1955; Kar and Mukherji, 1956; and others). The primary aim of such physiologic approach is to inhibit ovulation, fertilization or implantation or to influence some other sequential steps of the female reproductive cycle so that an evanascent sterile state is produced.

With such aim in view the author has undertaken a systematic search for agents either steroidal or non-steroidal, which would interfere with one or more of the above steps in the female reproductive cycle and thereby produce the desired temporary sterility.

The present report provides data on the weak androgenic compound 19-nortestosterone (19 NT). In addition to the possible anti-fertility properties, a detailed study of the general physiologic effects of this compound on the ovary and the uterus has been made. This has been considered necessary because of the paucity of knowledge concerning the physiological actions of 19 NT. Accordingly, the experiments dealing with the effects on the genital organs have been given a prior consideration in the text of this paper. Studies on the influence of 19 NT on the adrenal cortex have been reported elsewhere (Kar *et al.*, 1957; Kar and De, 1957).

B. EFFECT ON GENITAL ORGANS

Experimental Procedure

Animals : Sexually immature albino rats (one month old and with vaginal opening closed) of the Institute Colony were used in the study. In the ovariectomized animals 19 NT treatment started 10 days after the operation. The details of grouping of the animals for different experiments under this section are indicated in Table I. All the animals were maintained under uniform laboratory conditions throughout the period of investigation.

19-nortestosterone : For studies on its effects on the genital organs of intact (*Experiment I*) and ovariectomized rats (*Experiment II*) 3 dosages were used. The compound was injected by the subcutaneous route at the rate of 0.5, 1.5 and 3 mgs. (in sterile olive oil) daily for 10 days. This route of administration was followed in all of the subsequent experiments. The control animals received sterile olive oil alone in a similar manner.

Serum gonadotrophin: In *Experiment III* (Table I) a group of rats was injected first with 19 NT (3 mg. daily/rat) for 10 days, but from the 11th day it

was discontinued and serum gonadotrophin ('Antex' Dumex) was administered into the same animals by the intramuscular route (20 i.u. daily/rat) for another 7 days. A group of normal animals received serum gonadotrophin (20 i.u. daily/rat) alone in a similar manner for 7 days.

Cortisone : To test the effectiveness of 19 NT in preventing cortisone induced changes in the ovary, if any, a group of rats was injected subcutaneously with cortisone acetate ('Cortone' Merck, N.Y.) in a dosage of 1.5 mg. daily/rat for 10 days. Another group (Table I, *Experiment IV*) received 1.5 mg. of 19 NT *conjointly* with 1.5 mg. of cortisone (rat/day) for the same period.

To study the effect of cortisone pre-treatment (Table I. *Experiment V*) two groups of rats were first injected with 1.5 mg. of cortisone for 10 days and from the 11th day one group received 1.5 mg. of 19 NT *alone* for a further period of 10 days. The other cortisone treated group was left without any treatment for another 10 days. This experiment was a sequel to experiment IV and as such the two were initiated simultaneously.

Biochemical, histological and histochemical studies: All the experimental animals were sacrificed 24 hours after the final treatments. The ovary and the uterus were carefully dissected out, weighed to the nearest mg., and finally processed for biochemical and histochemical studies.

The total cholesterol content of the ovary was estimated colorimetrically by a modification (Roy, *et al.*, 1955) of a method by Zlatkis *et al.* (1953).

For histological and histochemical studies the tissues were fixed in 10 per cent neutral formalin or in chilled 80 per cent ethanol. For gross histology serial paraffin sections of formalin fixed tissues were stained with Ehrlich's hematoxylin followed by alcoholic eosin. The distribution of glycogen in the uterus was studied in similar sections by the periodic acid—Shiff (PAS) reaction (McMannus, 1948). Parallel sections were digested with saliva for 30 minutes at 37°C in order to ensure the accuracy of this test. Alkaline phosphatase was demonstrated in paraffin sections of ethanol fixed tissues by the technique of Gomori (1941) as laid down by Glick (1949). The sections were incubated in the substrate for 4 hours and were mounted without counterstaining.

Results

Experiment I. Effect of graded doses of 19 NT on the ovary and uterus of immature rats

(a) Ovary

Weight : It will be evident from Table I that the graded doses of 19 NT used in this study had no statistically significant effects on the absolute and relative ovarian weights.

Cholesterol : There was no significant change in the total cholesterol content of the ovary after treatment with graded doses of 19 NT (Table I).

Gross histology : Histological examination of the ovary of control animals revealed typical immature condition. The organ contained ovocytes and young follicles in different stages of growth. In some animals the beginning of antrum formation and thecal differentiation was visible in a few follicles. The corpora lutea were absent. The interstitium was entirely fibrocellular with poor vascularity (Plate V, fig. 1).

In the group of animals injected with 0.5 mg. of 19 NT the overall histological features were reminiscent of those of the controls. In a few animals, however, an accelerated rate of growth with evident thecal differentiation was noticeable

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TABLE I

Ovarian and uterine weights and cholesterol content of the ovary in different experimental animals

Expt. No.	Treatment	Mean weight of the ovary with S.E.		Mean weight of the uterus with S.E.		Mean cholesterol content of the ovary (Mg./gm. ovary) with S.E.	Mean body weight (gm.) with S.E.	
		Absolute (Mg.)	Relative (Mg./100 gm. body weight)	Absolute (Mg.)	Relative (Mg./100 gm. body weight)		Initial	Final
I.	Controls (Solvent only)	11.3± 2.02(8)*	21.4± 3.58(8)	15.6± 1.82(9)	28.2± 3.67(8)	24.3± 2.21(6)	45.8± 2.44(8)	50.2± 2.15(8)
	0.5 mg.–19 NT	15.0± 1.75(8)	31.0± 5.35(8)	70.2±10.10(8)	140.0±28.01(8)	25.9± 3.00(8)	41.1± 1.65(8)	54.5± 3.52(8)
	1.5 mg.–19 NT	14.6± 1.28(8)	24.8± 2.52(8)	103.2± 6.87(8)	175.8±20.07(8)	23.3± 2.11(8)	48.5± 1.58(8)	59.2± 2.21(8)
	3.0 mg.–19 NT	15.4± 2.83(8)	30.5± 5.17(8)	123.2± 6.53(8)	251.5±29.33(8)	22.2± 2.96(8)	41.3± 1.22(8)	49.5± 1.48(8)
II.	Ovariectomized (Solvent only)			11.3± 0.51(6)	23.9± 0.92(6)		43.2± 1.43(6)	47.2± 0.95(6)
	0.5 mg.–19 NT			84.9± 1.70(6)	170.2± 4.40(6)		47.5± 1.32(6)	50.0± 1.01(6)
	1.5 mg.–19 NT			110.6± 4.00(6)	237.5± 9.66(6)		42.8± 1.35(6)	46.8± 1.36(6)
	3.0 mg.–19 NT			118.8± 1.07(6)	240.4± 6.60(6)		45.6± 1.19(6)	49.5± 1.27(6)
III.	Controls (Solvent only)	15.7± 2.74(7)	24.4± 2.85(7)	16.8± 1.63(7)	28.7± 4.10(7)	28.8± 5.45(7)	52.7± 2.86(7)	59.4± 2.39(7)
	Serum gonadotrophin	73.8±15.83(7)	122.5±22.00(7)	139.7±13.61(7)	232.9±17.71(7)	10.4± 1.45(7)	50.7± 0.77(7)	60.1± 0.82(7)
	Serum gonadotrophin plus 19 NT	70.2±10.43(7)	110.2±14.83(7)	141.4±11.46(7)	231.9±29.95(7)	11.3± 1.38(7)	42.4± 1.78(7)	63.1± 1.27(7)
IV.	Controls (Solvent only)	11.5± 1.68(8)	25.6± 3.00(8)	42.4± 5.34(8)	70.4±16.03(8)	17.4± 3.70(7)	33.3± 1.95(8)	43.8± 2.50(8)
	Cortisone	8.6± 1.48(8)	25.3± 5.59(8)	25.5± 4.18(8)	61.1±13.84(8)	15.7± 2.09(8)	32.3± 0.86(8)	34.2± 1.06(8)
	Cortisone plus 19 NT	12.9± 2.88(7)	32.4± 6.47(7)	126.8±10.28(7)	306.8±49.74(7)	20.7± 3.99(7)	33.0± 1.10(8)	34.5± 1.14(8)
V.	Controls** (Solvent only)	11.5± 1.68(8)	25.6± 3.00(8)	42.4± 5.34(8)	70.4±16.03(8)	17.4± 3.70(8)	33.3± 1.95(8)	43.8± 2.50(8)
	Cortisone (*Pretreatment*)	8.9± 1.58(6)	20.6± 4.74(6)	22.4± 4.14(7)	46.7± 5.62(7)	14.6± 2.41(6)	33.8± 1.31(8)	45.2± 2.27(7)
	Cortisone plus 19 NT (*Pretreatment*)	9.5± 2.07(6)	22.3± 5.58(6)	127.4± 4.59(7)	345.5±36.36(7)	7.6± 1.85(6)	28.6± 1.80(6)	40.7± 1.06(7)

* Figure in parenthesis indicates the number of animals.
** Data from experiment III.

in some follicles. Nevertheless, this condition was not consistently observed in this group. The corpora lutea were absent and the features of the interstitium were virtually similar to those of the controls.

Histological indications of stimulation of follicular growth were visible in the ovary of many animals of the group which received 1.5 mg. of 19 NT daily. Mature Graafian follicles and corpora lutea were also present in some ovaries (Plate V, fig. 2). The entire organ was hyperaemic.

The highest dose of 19 NT (3 mg. daily) seemed to accelerate follicular growth and maturation. Ripe Graafian follicles and corpora lutea were present in many cases. Due to growth and maturation of the follicles the fibrocellular interstitium appeared to be considerably obliterated in some cases. Marked hyperaemia of the organ was evident.

It should be noted that the stimulation of follicular growth by 19 NT at the higher dose levels (1.5 and 3 mgs.) was not consistent because in some animals the effect was not quite so marked. The corpora lutea were also not generally present.

Alkaline phosphatase : In agreement with the findings of Dempsey *et al.*, (1949), Talmage (1949), Kamell and Atkinson (1948), Kar (1953) and others, it was noticed that in the control animals only the nucleus of the ovocytes had given a weak positive reaction for alkaline phosphatase. The developing follicles, however, contained large amounts of the enzyme in the granulosa cells, but the distribution in the ovum appeared to be faint and patchy (Plate V, fig. 3). The cellular elements and the endothelium of the vascular sinusoids in the interstitium showed intense phosphatase activity.

The pattern of distribution of alkaline phosphatase in the ovary of animals injected with 0.5 mg. of 19 NT daily was more or less similar to that of the controls, but the overall concentration seemed to be somewhat higher. This was evident from the more intense staining reactions of the interstitial elements. The theca of the more developed follicles also showed a heavy mobilization of the enzyme.

The concentration of alkaline phosphatase was considerably high in the group which received 1.5 mg. of 19 NT daily. This was seen from the pronounced enzyme activity in the granulosum and the theca of the follicles. The corpora lutea also contained considerable amounts of the enzyme, particularly in the nucleus of the lutein cells. The endothelium of the vascular sinusoids in the corpora lutea gave strong positive reactions for the enzyme. The overall concentration was higher than that of the controls or the group which received 0.5 mg. dose daily.

In contrast to the above two groups, the administration of 3 mg. of 19 NT daily caused a reduction in the concentration of alkaline phosphatase in the ovary. This was clear from the rather weak reactions in practically all the elements of the ovary. The cellular elements of the interstitium gave only moderate reactions in the nucleus, but the cytoplasm was virtually negative. The endothelium of the vascular sinusoids in the interstitium also showed reduced enzyme activity. The follicles were no exception either as they gave less intense staining reactions than in the other groups (Plate V, fig. 4).

(b) Uterus

Weight : It will be seen from Table 1 that graded doses of 19 NT caused a significant increase ($P < .001$) in the absolute weight of the uterus. From the mean weights of the organ it would also appear that this increase was proportional to the dosage. However, a comparison of the mean uterine weights of 0.5 mg. and 1.5 mg. groups showed that the difference was statistically significant ($P < .02$); but a similar comparison between 1.5 mg. and 3 mg. groups revealed that the difference between the means was insignificant.

The relative weight of the uterus showed similar changes after 19 NT treatment. Thus, the graded doses caused a significant increase in the uterine weight ($P < .001$) and the means were increasingly higher in proportion to the dosage (Table I). Nevertheless, test of significance showed that only the mean uterine weight of the 3 mg. group was significantly higher than that of 0.5 mg. group ($P < .02$); but the difference between the means of 0.5 mg. and 1.5 mg. groups was insignificant. Similarly, the mean uterine weights of 1.5 mg. and 3 mg. groups were statistically insignificant.

19 NT treatment caused premature opening of the vagina and the time taken for this to happen in 100 per cent of the experimental animals was dependent on the dosage. Thus, in 0.5 mg. group the mean time taken was 120 hours from the commencement of injections, but in 1.5 mg. group it was reduced to 96 hours. The highest dose (3 mg.) caused opening of the vagina within 72 hours from the institution of hormone regimen. The vaginal opening of all of the control animals, however, remained closed throughout thé experimental period.

The examination of vaginal smears of animals injected with 19 NT showed a great deal of mucous and mixed type of cells. A typical oestrus smear containing only cornified cells was absent.

Gross histology : The uterus of control animals presented a typical infantile appearance and the vascularity was poor. The mucosal epithelium was of the low columnar type and the stroma consisted principally of the fibroblasts and occasional leucocytes. The serosa and the muscularis were thin and inconspicuous. The endometrial glands were few and the cavum was small and slit-like (Plate V, fig. 5).

In the 0.5 mg. group the increase in uterine weight was ascribable histologically to the growth of the muscularis and the serosa. The features of the endometrium were not unlike those of the controls. An increased vascularity was, however, evident and the cavum appeared to be somewhat tortuous.

Pronounced growth of the muscularis and the serosa was noticeable in the uterus of the group which received 1.5 mg. of 19 NT daily. The endometrium also showed marked growth and the glands tended to be well developed. The mucosal epithelium exhibited the typical apical and basal arrangement of the nucleus and frequent vacuolar degeneration characteristic of a mature animal. The cavum was prominent and tortuous. The entire organ was oedematous and hyperaemic.

The highest dose of 19 NT (3 mg.) caused pronounced oedema and hyperaemia. The serosa and muscularis showed marked growth. The endometrium was considerably thickened and contained many glands in full secretory state. The epithelium showed frequent vacuolar degeneration. The cavum was tortutous owing to the 'lacing' nature of the endometrium (Plate V, fig. 6). In one animal endometrial cysts were seen in the uterus.

Alkaline phosphatase : In the controls the serosa and the muscularis were virtually devoid of phosphatase activity. However, the endothelium of the vascular sinusoids in these areas gave positive reactions for the enzyme. In the endometrium the epithelium gave weak positive reactions both in the nucleus and in the cytoplasm. The stromal connective tissue cells contained small amounts of phosphatase, but the endothelium of the sinusoids was strongly reactive. The few immature glands present in the endometrium gave weak positive reactions (Plate V, fig. 7).

The pattern of distribution of alkaline phosphatase in the uterus of the group injected with 0.5 mg. of 19 NT daily was the same as in the controls, but the overall concentration appeared to be a little higher. This was evident from intense staining reactions of the epithelium and the endometrial glands.

In the animals injected with 1.5 mg. of 19 NT daily the distribution and concentration of alkaline phosphatase in the uterus were more or less similar to those

of the 0.5 mg. group. Nevertheless, the glandular secretions in the endometrium contained considerable amounts of the enzyme.

In the 3 mg. group there was a strong mobilization of alkaline phosphatase in the endometrium. This was noticeable in all the elements of the endometrium, both cellular and vascular. The glandular secretions contained considerable amounts of the enzyme. The endothelium of the sinusoids in other areas also showed marked phosphatase activity (Plate V, fig. 8).

Glycogen : The uterus of the control animals contained very little glycogen as shown by the PAS reaction (Plate V, fig. 9).

In general the concentration of glycogen in the uterus of the group injected with 0.5 mg. of 19 NT daily was more or less similar to that of the controls. In some epithelial cells of the mucosa, however, a patchy localization of glycogen was noticeable.

1.5 mg. dose favoured a mobilization of glycogen in the uterus. This was noticeable particularly in the mucosal epithelium and the glands. In the latter glycogen was present in the epithelium as well as in the secretions.

In the group injected with 3 mg. of 19 NT daily the contentration of glycogen appeared to be the highest. This substance was mainly localized in the epithelium and in the glandular secretions. In certain regions of the muscularis patchy staining reactions for glycogen were also visible (Plate VI, fig. 10).

Experiment II. Effect of graded doses of 19 NT on the uterus of immature ovariectomized rats

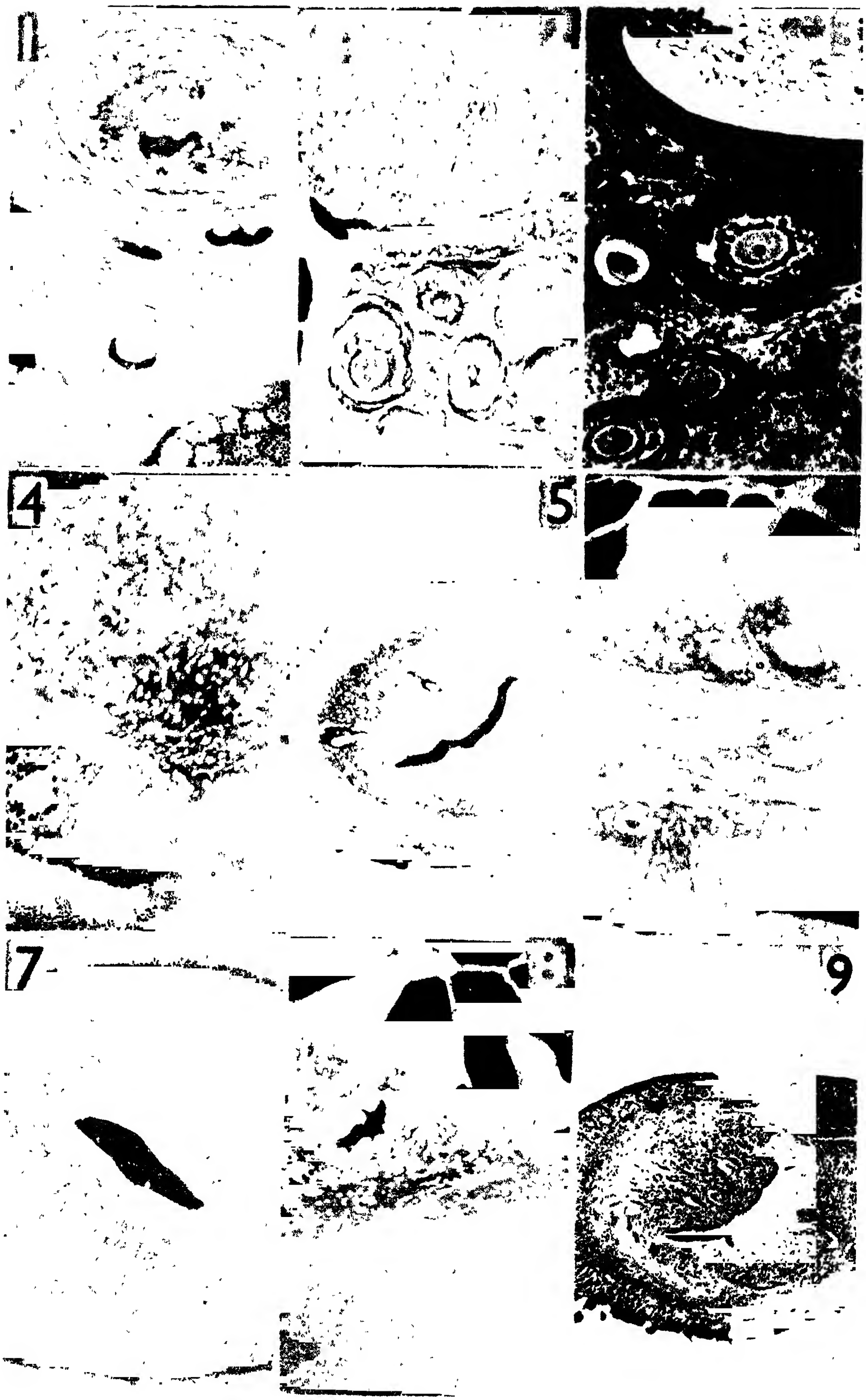
Weight : It will be clear from Table I that graded doses of 19 NT caused a significant increase ($P < .001$) in the absolute and relative weights of the uterus of immature ovariectomized rats. The increase in absolute weight was proportional to the dosage, but this was not the case with the relative weight of the organ. A comparison of the mean relative uterine weights of 0.5 mg. and 1.5 mg. groups showed that the difference was statistically significant ($P < .001$); but a similar evaluation between 1.5 mg. and 3 mg. groups revealed that the difference in the mean relative weights of the uterus was statistically insignificant.

As with the intact immature animals, 19 NT administration to ovariectomized immature animals caused premature opening of the vagina. The mean time taken for such opening in 100 per cent of the treated animals was dependent on the dosage and the actual figures for 0.5, 1.5 and 3 mg. dosages were 120, 96, and 72 hours respectively. These figures were comparable to those obtained with the intact immature rats and indicated that with the increasing dosage levels the time taken for the vagina to be patent was reduced. The vaginal opening of all of the untreated ovariectomized animals was, however, closed throughout the experimental period.

The examination of vaginal smears of ovariectomized animals injected with 19 NT showed much mucous and mixed type of cells, but a typical oestrus smear was not encountered.

Gross histology : The histological features of the uterus of untreated ovariectomized animals were similar to those of the uterus of intact immature animals (Plate V, fig. 5). Likewise, the histological details of the uterus of ovariectomized animals injected with 0.5, 1.5 and 3 mg. dosages were comparable to those of the uterus of intact animals subjected to 19 NT treatment at identical dosages (Plate V, fig. 6).

Alkaline phosphatase : The distribution and concentration of alkaline phosphatase in the uterus of untreated ovariectomized animals were similar to those of the uterus of intact control animals (Plate V, fig. 7). The increase in alkaline phosphatase activity in the uterus of 19 NT treated animals was comparable to a similar increase in the uterus of intact animals injected with the hormone (Plate V, fig. 8).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Glycogen: The uterus of untreated ovariectomized animals contained very little glycogen (Plate V, fig. 9), but 19 NT treatment caused a deposition of glycogen in a manner comparable to the mobilization of this substance in the uterus of intact animals under the influence of this compound (Plate VI, fig. 10).

Experiment III. Effect of 19 NT pre-treatment on the response of the ovary and the uterus of immature rats to serum gonadotrophin

(a) Ovary

Weight: Serum gonadotrophin caused a significant increase in both the absolute and relative weights of the ovary ($P < .001$). This was true whether the animals were pretreated with 19 NT or not (Table I). The difference in absolute and relative ovarian weights between the two groups of animals injected with serum gonadotrophin either alone or after pretreatment with 19 NT was, however, statistically insignificant.

Cholesterol: In agreement with our previous findings (Kar *et al.*, 1954) it was noticed that serum gonadotrophin caused a significant fall in total cholesterol content of the ovary ($P < .01$) of normal animals. 19 NT pretreatment did not interfere with a similar drop in ovarian cholesterol content ($P < .01$). There was, however, no significant difference in the cholesterol concentration of the ovary between the two serum gonadotrophin treated groups (Table I).

Gross histology: The histological details of the ovary of control animals were practically similar to those of the ovary of controls in experiment I (Plate V, fig. 1). An immature condition was evident from the presence of exclusively young and undifferentiated ovocytes and follicles. On an average, the follicular growth did not reach beyond the beginning of antrum formation. The corpora lutea were absent and the interstitium was fibrocellular with scant vascularity.

Histological indications of stimulation of ovarian growth after serum gonadotrophin administration were discernible from the rapid rate of maturation of the available follicles and bulky luteinization. Very few young follicles were seen in the ovary. Follicular atresia and haemorrhage in the follicles were common. Haemorrhagic corpus luteum was also present. There was pronounced hypertrophy of the interstitial cellular elements and a hyperaemic condition was readily recognizable. Many follicles appeared to be spent and devoid of ova (Plate VI, fig. 11).

Histological details of the ovary of 19 NT pre-treated animals subsequently injected with serum gonadotrophin were similar to those of the ovary of animals which received serum gonadotrophin alone. There was accelerated rate of maturation of the follicles and bulky luteinization. Haemorrhagic corpora lutea and follicles were frequently seen and follicles devoid of ova were also common. The interstitium presented comparable features.

Alkaline phosphatase: The distribution and concentration of alkaline phosphatase in the ovary of control animals were similar to those of the ovary of the previous controls (Plate V, fig. 3).

The overall concentration of alkaline phosphatase in the ovary of serum gonadotrophin treated animals was higher than that in the controls. This seemed to be related to follicular maturation and bulky luteinization. The pattern of distribution of the enzyme in the different elements of the ovary was, however, not different from that of the controls (Plate VI, fig. 12).

The distribution and concentration of alkaline phosphatase in the ovary of animals injected with serum gonadotrophin after 19 NT pre-treatment was practically similar to those of the animals which received serum gonadotrophin alone.

(b) Uterus

Weight : It will be seen from Table I that both the absolute and the relative weight of the uterus increased significantly after serum gonadotrophin injections ($P < .001$). Such increase occurred in animals which had received serum goan-dotrophin either alone or after pre-treatment with 19 NT. The difference in absolute and relative uterine weights in the two serum gonadotrophin treated groups was, however, insignificant.

Gross histology : The histological details of the uterus of control animals were similar to those described for the controls in experiment I (Plate V, fig. 5).

In animals injected with serum gonadotrophin alone marked stimulation of the uterus was evident upon histological examination. The entire organ was hyperaemic and oedematous. There was considerable growth of the serosa, muscularis and the endometrium. The latter contained well-developed glands and showed typical 'lacing' patterns. The features of the epithelium were similar to those of the mucosal epithelium of the 3 mg group (Plate VI, fig. 13). It should be noted that serum gonadotrophin treatment caused opening of the vagina within 72 hours.

The histological features of the uterus of animals injected with serum gonadotrophin after 19 NT pre-treatment were similar to those of the animals which received serum gonadotrophin alone.

Alkaline phosphatase : The distribution and concentration of alkaline phosphatase in the uterus of the control animals were similar to those of the uterus of control animals in experiment I (Plate V, fig. 3).

Serum gonadotrophin treatment elevated the concentration of alkaline phosphatase in the uterus. This seemed to be particularly noticeable in the endometrium. Thus, the vascular and the cellular elements of the stroma contained considerable amounts of the enzyme. The epithelium and the secretion of the glands showed marked phosphatase activity. The endothelium of the vascular sinusoids in the muscularis and the serosa also gave intense staining reactions for the enzyme (Plate VI, fig. 14).

The distribution and concentration of alkaline phosphatase in the uterus of animals which received serum gonadotrophin after 19 NT pre-treatment were similar to those of the animals treated with serum gonadotrophin alone.

Glycogen : The uterus of the control animals contained very little glycogen (cf. Plate V, fig. 9).

Serum gonadotrophin favoured a deposition of glycogen in the uterus but not to the same extent as in 19 NT treated animals (1.5 mg. and 3 mg. groups, *Experiment I*). This could be gauged from the relatively low concentration of glycogen in the endometrium and in the glands (Plate VI, fig. 15).

Serum gonadotrophin administration to 19 NT pre-treated animals caused greater deposition of glycogen in the uterus as compared to the above. The picture was more or less similar to that seen after treatment with 19 NT alone (cf. Plate VI, fig. 10).

Experiment IV. Effect of simultaneous administration of cortisone and 19 NT on the ovary and uterus of immature rats

(a) Ovary

Weight : The absolute and relative weights of the ovary of cortisone or cortisone plus 19 NT treated groups did not differ significantly from those of the controls (Table I). The difference in the mean absolute and relative ovarian weights between the two hormone treated groups was likewise statistically insignificant.

Cholesterol : It will be seen from Table I that the total cholesterol content of the ovary was also not influenced in a significant manner after cortisone or cortisone plus 19 NT treatments.

Gross histology : The histological features of the ovary of control and cortisone treated animals were similar and were comparable to those of the controls in experiment I. There were, however, indications of stimulation of ovarian growth in some animals of the group which received 19 NT and cortisone conjointly. These were evident from the more advanced stages of growth of the follicles and formation of corpora lutea (Plate VI, fig. 16) An increased vascularity was also evident.

Alkaline phosphatase : The distribution and concentration of alkaline phosphatase in the ovary of control and cortisone treated animals were virtually similar to those of the controls in experiment I. In the group which received the two hormones conjointly the overall concentration of the enzyme, however, seemed to be somewhat low. This was due to weak reactions in the corpora lutea and in the interstitial cellular elements (Plate VI, fig. 17).

(b) Uterus

Weight : Cortisone treatment caused a significant decrease only in the absolute weight of the uterus ($P < .05$) but not in its relative weight. Simultaneous treatment with cortisone and 19 NT, however, stimulated the uterine weight, both absolute and relative, in a highly significant manner ($P < .001$).

Gross histology : Histological characteristics of the uterus of the control and cortisone treated animals were more or less similar to those of the controls in experiment I. Simultaneous administration of cortisone and 19 NT caused oedema and hyperaemia. There was marked growth of the serosa, muscularis and the endometrium. The glands were well developed and the mucosal epithelium presented features comparable to those of a mature animal. The overall picture was not unlike those of the uterus of the group which received 1.5 mg. of 19 NT.

Alkaline phosphatase : The distribution and concentration of alkaline phosphatase in the uterus of control and cortisone treated rats were similar to those of the uterus of controls in experiment I (Plate V, fig. 7). After simultaneous administration of cortisone and 19 NT there was an overall increase in concentration of the enzyme in the uterus. The glandular secretions in the endometrium contained considerable amounts of phosphatase. The pattern of distribution of the enzyme was comparable to those of the uterus of the group injected with 1.5 mg. of 19 NT alone.

Glycogen : Cortisone treatment did not cause any change in the concentration of glycogen in the uterus. Simultaneous treatment with cortisone and 19 NT favoured a deposition of glycogen in the uterus. The overall concentration and the pattern of distribution of glycogen in this group were similar to those of the uterus of animals injected with 1.5 mg. of 19 NT alone.

Experiment V. Effect of cortisone pre-treatment on the response of the ovary and the uterus of immature rats to 19 NT

(a) Ovary

Weight : Cortisone treatment caused a significant loss only in the absolute ovarian weight ($P < .01$) but no significant change in the relative weight of the ovary was noticeable (Table I). 19 NT administration to cortisone pre-treated rats failed to provoke any significant change in the absolute and relative ovarian weights. In the two hormone treated groups, however, the difference in mean ovarian weights, both absolute and relative, was statistically insignificant.

Cholesterol : Cortisone treatment (Table I, *Experiment V*) did not elicit any significant change in total cholesterol content of the ovary. 19 NT given to cortisone pre-treated animals, however, caused a significant fall in cholesterol concentration of the ovary ($P < .05$). The difference in mean cholesterol content of the ovary of the two hormone treated groups was also statistically significant ($P < .05$).

Gross histology : The histological features of the ovary of cortisone treated rats were similar to those of the controls in experiment I (Plate V, fig. 1). In the group which received 19 NT after cortisone pre-treatment the histological appearance of the ovary was practically similar to that of the animals which received 1.5 mg. of 19 NT alone.

Alkaline phosphatase : No change in the distribution and concentration of alkaline phosphatase was seen in the ovary of the cortisone treated group. The pattern was similar to that of the controls in experiment I (Plate V, fig. 3). In the cortisone pre-treated group injected subsequently with 19 NT the enzyme activity in the ovary was practically similar to that in the animals injected with 1.5 mg. of 19 NT alone.

(b) Uterus

Weight : The absolute uterine weight in the cortisone treated group was significantly less ($P < .02$) as compared to that of controls but the relative weight of the organ did not show any significant change. These were comparable to the response in ovarian weights noticed after cortisone treatment. 19 NT injections to cortisone pre-treated animals, however, caused a significant increase both in absolute and relative weights of the uterus ($P < .001$).

Gross histology : Cortisone treatment did not cause any change in the histology of the uterus. The features were similar to those of the controls in experiment I (Plate V, fig. 5). The histological characteristics of the uterus were in no way different from those of the group treated with 1.5 mg. of 19 NT alone.

Alkaline phosphatase : There was no change in the distribution and concentration of alkaline phosphatase after cortisone treatment. The pattern was similar to that of the control animals in experiment I (Plate V, fig. 7). Administration of 19 NT to cortisone pre-treated animals caused a slight rise in concentration of the enzyme in the uterus as compared to the group which received 19 NT alone. This was ascribable to more intense reactions in the epithelium (Plate VI, fig. 18), but otherwise the distribution of phosphatase was similar to that of the uterus of the 1.5 mg. group.

Glycogen : The concentration of glycogen in the uterus did not show any change after cortisone treatment. In the group which received 19 NT after cortisone pre-treatment the concentration of glycogen was similar to that in the group injected with 1.5 mg. of 19 NT alone.

C. Effect on Pregnancy

Experimental Procedure

Animals : Sexually mature albino rats of the Institute Colony weighing 156 to 200 gm. were used in this study. The males were of proven fertility. Sexually immature females (45 to 50 gm.) were a month old and their vaginal opening was closed without exception.

Examination of vaginal smears : Before commencement of 19 NT treatments the vaginal smears of all of the mature females were examined daily for 3 complete oestrus cycles in order to ensure their normal recurrence. An animal showing any irregularity of the cycle was promptly discarded. The average length of the oestrus cycle was found to be 4.5 days.

The subsequent daily examination of vaginal smears started on the day on which 19 NT regimen was instituted and continued throughout the rest of the treatment period.

The vaginal smears were taken once a day at 11 o'clock in the morning.

19 NT administration : 19 NT was given either by *subcutaneous injection* or *orally*. In the latter route the drug was introduced into the lower portion of the oesophagus by a blunt feeding needle.

19 NT was dissolved either in sterile olive oil or in propylene glycol. The control animals received the same volume of these solvents in similar manners.

Mating : For mating two females were caged with one male. The day on which a vaginal smear contained spermatozoa was considered as the day of mating. The males were frequently changed during mating in order to avoid the possibility of variations in fertility. About 13 to 16 days after cohabitation was started and every 2 to 3 days thereafter, the females were palpated and weighed for detection of pregnancy. The mating test period was kept constant (60 days) in all of the experiments irrespective of the time of commencement and duration of 19 NT treatments. However, in experiments where it was deemed necessary to re-mate the animals after withdrawal of 19 NT treatments no such limited test period was employed.

Experiment I. 19 NT administration to sexually immature female rats and its effect on pregnancy

Sexually immature female rats were injected with 19 NT (3 mg./rat/every alternate days) for 24 days (a total of 12 injections over 24 days). At the end of the treatment period the vaginal openings which were closed at the beginning of the experiment, were all patent. Daily examination of vaginal smears for 10 days subsequent to the cessation of hormone treatment showed the presence of mucous and mixed type of cells. It was difficult to categorize the smears in terms of the classic stages of the oestrus cycle. There was no cyclicity in the vaginal smear picture at least during the 10 days examination period.

The treated animals were mated when they were 105 days old. At this age the vagina of all the control animals were found to be open and the examination of vaginal smears of these and 19 NT treated animals showed cyclic recurrence of oestrus. The results of mating are presented in Table II.

It will be seen from Table II (*Experiment I*) that 19 NT treatment for 24 days during the prepubertal period did not interfere with the subsequent attainment of sexual maturity or conception in rats. There was no difference between the controls and the hormone treated animals with regard to the parameters employed in this study for the evaluation of effect of 19 NT.

Experiment II. Effect of 19 NT treatment to adult female rats before mating on pregnancy

Adult female rats were injected with 19 NT (3 mg./rat/every alternate days) for 24 days (a total of 12 injections over 24 days) and on the 25th day they were caged with the males. Examination of vaginal smears during the treatment period showed irregularities of the oestrus cycle which, however, tended to disappear during the mating period. The irregularities of the vaginal smears consisted mainly of the occurrence of mixed smears during the oestrus as a result of which it became difficult to define this stage in the usual clear-cut manner. The vaginal smears during the post-mating period were of the dioestrus type. A similar condition of the vaginal smear was seen in the control animals after mating.

It will be evident from Table II that 19 NT administration to adult female rats *before mating* had no effects on conception as 100 per cent of the animals became pregnant within the 60 days test period. The time of mating, the number of young per litter and the percentage of the dead young were similar to those of the controls.

TABLE II

Effect of 19 NT on mating and conception in rats

Expt. No.	Treatment	Days between cohabitation and mating		Percent conceived within 60 days	Average no. of live young per litter	Dead young (Percentage of total young)
		Mean	Range			
I.	Controls (10)*	4.0	(1– 6)	100	7.8	1.92
	19 NT-*during prepuberal condition* (10)	3.5	(1– 7)	100	8.0	2.00
II.	Controls**(12)	3.1	(1– 6)	100	8.4	1.94
	19 NT-*before mating* (12)	3.4	(1– 7)	100	8.1	2.00
III.	19 NT-*before and during mating* (12)	5.7	(5– 7)	100	8.5	1.92
IV.	19 NT-*during mating* (12)	6.1	(5– 8)	100	0.08	2.02
	Controls***(10)	3.0	(1– 5)	100	9.0	1.3
V.	19 NT–*Subcutaneous injection during mating* (10)	6.2†	(3–11)	nil	nil	nil
VI.	19 NT–25 *mg. twice a a month orally during mating* (10)	4.5	(1– 7)	100	8.9	1.1
VII.	19 NT–100 *mg. once a month orally during mating* (10)	2.1	(1– 4)	100	8.5	nil

* Figure in parenthesis indicates the number of animals.
** Common controls for experiments II–IV.
*** Common controls for experiments V–VII.
† Only 6 out of 10 animals mated.

Experiment III. Effect of 19 NT treatment to adult female rats before and during mating on pregnancy

Adult female rats were injected with 19 NT (3 mg./rat/every alternate days) first *before mating* for 12 days (6 injections over 12 days) and then for a further period of 12 days *during mating* (6 injections over 12 days). The hormone treatments before and during mating were without any break so that the compound was administered continuously during a total period of 24 days. The males were introduced into the cages on the 13th day from the beginning of injections. Examination of vaginal smears during the pre-mating treatment period showed irregularities which persisted till mating after which an universal dioestrus condition was noticed in the smears. The nature of irregularities of the vaginal smears

consisted mainly of the absence of typical oestrus picture with 100 per cent cornified cells, but otherwise the cyclicity was virtually undisturbed.

Table II (*Experiment III*) will show that 19 NT treatment *before and during mating* had no effects on conception as 100 per cent of the animals became pregnant during the test period. The average number of young per litter and the percentage of dead young were almost similar to those of the controls. Nevertheless, the course of treatment followed in this experiment caused a delay in mating. This was evident from the fact that mating occurred in this group (Table II, *Experiment III*) on an average of 5.7 days from cohabitation, whereas in the controls the mating occurred on the average of 3.1 days. The difference was highly significant ($P < .001$).

Experiment IV. Effect of 19 NT treatment to adult female rats during mating on pregnancy

Adult female rats were injected with 19 NT (3 mg./rat/every alternate days) for a period of 24 days *during mating*. The females were caged with the males 6 hours after the commencement of injections. The examination of vaginal smears showed irregularities of oestrus till mating after which the smears became exclusively of the dioestrus type. The nature of irregularities was, however, the same as seen in experiment III.

A perusal of Table II (*Experiment IV*) will indicate that except causing a delay in mating 19 NT had no effects on pregnancy. The average number of young per litter and the percentage of dead young were practically similar to those of the controls. The delay in mating was, however, interesting as the hormone-treated animals mated on an average of 6.4 days from cohabitation. This figure was significantly longer ($P < .001$) than the corresponding figure for average mating period in the controls (3.1 days).

The results obtained in experiments II and IV clearly indicated that the delaying effect of 19 NT on mating was noticeable *only if the injections were given during the mating period*. Accordingly, in the subsequent experiments the compound was administered during the mating period. The males were introduced into the cages 6 hours after the commencement of 19 NT treatments.

Experiment V. Effect of prolonged 19 NT treatment to adult female rats during mating on pregnancy

Adult female rats were injected with 19 NT (3 mg./rat/every alternate days) continuously for 60 days. A total of 30 injections of the hormone were given during this period. Examination of vaginal smears showed irregularities within a few days after commencement of 19 NT administration. The nature of irregularities was, however, the same as noted in experiments III and IV. Such irregularities persisted for about a month after which a dioestrus condition ensued lasting for the remainder of the treatment period. The control animals eventually passed into a dioestrus condition after mating.

By the criterion employed for detection of mating it was seen that only 60 per cent of the animals mated, but the rest did not mate at all during the test period of 60 days. Those animals which mated showed a delay in mating as they mated on an average of 6.2 days from cohabitation, whereas the corresponding figure for controls was 3.0 days. The difference was statistically significant ($P < .01$).

None of the treated animals conceived during the test period of 60 days ; nor was there any overt signs of abortion. In contrast 100 per cent of the control animals became pregnant within this period.

In order to examine whether the conception inhibiting effects of 19 NT as seen in this group were temporary or permanent, the animals were kept under constant observation after the termination of injections from the 61st day. They were, however, caged with the same males and the daily examination of vaginal smears continued as before. The pseudopregnant condition induced by 19 NT lasted for an appreciable period of time even after the discontinuation of treatments. During this period no mating occurred. It took on an average 42.8 days (*range* 30 to 52 days) for the recurrence of cyclic oestrus and mating in all of the animals including those which did not mate at all during the treatment period. At the time of writing of this paper, that is 90 days after the discontinuation of injections, 70 per cent of the animals became pregnant and gave birth to normal young ones.* The pregnant animals included the same ones which did not mate during the treatment period. The average number of young ones per litter was 8.5 and the percentage of dead young was 1.5. These figures compared favourably with similar figures for other experimental groups (Table II).

Experiment VI. Effect of oral administration of 19 NT (twice a month) to adult female rats on pregnancy

Adult female rats were administered 25 mg. of 19 NT orally twice a month for 60 days. There was no interference with the oestrus cycle due to the administration of the drug ; the animals eventually passed into a dioestrus condition after mating.

It will be seen from Table II that such feeding of 19 NT had no effect on pregnancy as 100 per cent of the animals became pregnant during this period. There were also no significant effects on mating, the average number of young per litter and the percentage of dead young.

Experiment VII. Effect of oral administration of 19 NT (once a month) to adult female rats on pregnancy

Adult female rats were given 100 mg. of 19 NT orally once a month for 60 days. The oestrus cycle continued in the normal manner till mating after which the smears eventually became of the dioestrus type.

Oral administration of 19 NT did not interfere with mating or pregnancy as all of the experimental animals conceived within the test period. There was also no significant effect on the average number of young per litter or percentage of dead young.

C. Discussion

Effect of 19 NT on the ovary

The androgens are known to have specific effects on the ovary. Thus, in the immature rats a single injection of 1 to 10 mg. of testosterone propionate (TP) leads to follicular maturation during the ensuing 3 to 8 days. This may be followed by luteinization without ovulation (Nathanson *et al.*, 1938 ; Salmon, 1939 ; Noble, 1939 ; and others). In adult rats, on the other hand, the administration of TP in daily doses of 1 to 5 mg. for several weeks tends to increase the number and diameter of the follicles, but the interstitial cellular growth is diminished ; the net effect is a gross reduction in weight of the ovary (Aschheim and Varangot, 1939 ; Korenchevsky and Hall, 1940). The luteal reaction in such animals is also

* *Note added to the proof.* Eventually by 125 days 100 per cent of the animals became pregnant.

influenced by TP, but the nature of this reaction seems to be determined by the stage of the oestrus cycle at which the hormonal regimen is instituted (Wolfe and Hamilton, 1937 ; Laqueur and Fluhmann, 1942). Prolonged treatment with TP over a period of one or more months almost invariably causes ovarian atrophy. The follicular maturation and ovulation cease with the resultant abolition of the sexual cycle (Wolfe and Hamilton, 1937 ; Parkes and Zuckerman, 1938 ; Laqueur and Fluhmann, 1942). These effects are not restricted to rats, but have been observed in other species as well (Burrows. 1949 ; Kar, 1948 ; Dorfman and Shipley, 1956). Alterations in the pituitary gonadotrophin output in one manner or another by the androgens have been implicated as the root cause of such changes in the ovaries.

Viewed against such a clear background, the response of the ovary of immature rats to 19 NT would appear somewhat anomalous. Thus, the graded doses of 19 NT used in the present study do not evoke any significant change in the weight of the ovary. Similarly, the cholesterol content of the ovary fails to respond to 19 NT administration in increasingly higher doses. In contrast to these, the histological indications of stimulation of follicular growth are present in the ovary of many of the hormone-treated animals ; in others the follicular response is not quite so marked. The corpora lutea are also present in the ovary of a few animals subjected to the higher dose levels (1.5 to 3 mg.). In the control animals, however, the follicles are invariably immature and the corpora lutea are universally absent. It is interesting that the overall activity of alkaline phosphatase in the ovary increases proportionately in 0.5 and 1.5 mg. dosage groups, but decreases in the 3 mg. group.

Such erratic response of the ovary to 19 NT is difficult to explain. It would appear that in those animals which show indications of follicular maturation and corpora lutea formation. 19 NT stimulates pituitary gonadotrophic activity. If the androgenicity of this compound is taken into consideration (Kar *et al.*, 1957) such effects on the ovary *via* the pituitary will be entirely within the limits of expectation. Reports of a similar nature regarding the gonadotrophic influence of androgens on the ovary are also not uncommon (*vide supra*). But the obivous difficulty arises in considering those cases in a particular dosage group which do not show any response to 19 NT. It has been observed that the reactivity of the ovary to androgens particularly the luteal response is influenced by the stage of the oestrus cycle at which the hormone is administered (Wolfe and Hamilton, 1937 ; Laqueur and Fluhmann, 1942). This criterion, however, cannot be invoked to explain the variable nature of ovarian response obtained in the present study as only sexually immature animals are involved. It may be that the ovary of those animals which respond to 19 NT are already on the way to maturity and as such reacts readily to the extra gonadotrophic stimulus provided by this compound. In others the ovary is perhaps relatively less advanced as regards maturity or is in a completely immature condition ; and one of these causes could be held responsible for this peculiar refractoriness to 19 NT. Such explanations, however, tacitly assume the primacy of the rôle of the pituitary in mediating any action of 19 NT on the ovary, besides, of course, emphasizing the importance of the actual state of the pituitary-ovarian axis at the commencement of androgen administrations, in determining the overall responsiveness of the ovary of otherwise immature animals. To what extent such remarks are justified will depend entirely on future experimentation, but in the author's opinion the state of pituitary-ovarian axis is a moot point.

In contrast to the anomalous effects of graded doses of 19 NT, the response of the ovary to serum gonadotrophin after pre-treatment with the androgen seems more clear-cut. This is evident from the fact that the ovary of animals which received the gonadotrophic hormone alone shows indications of precocious stimu-

lation as judged by the various criteria employed in this study. By the same criteria again, it is seen that treatment with 19 NT does not interfere with the action of serum gonadotrophin on the ovary ; the latter evokes typical stimulatory responses and 19 NT does not hinder such responses.

In course of the author's previous studies on the physiological properties of 19 NT it was noticed that it antagonized the typical anti-adrenal effects of cortisone, but did not interfere with the antiphlogistic effects of the latter (Kar *et al.*, 1957; Kar and De, 1957). Cortisone has been reported to cause an increase in ovarian weight in sexually immature rats due to accelerated follicular development (Blivaiss *et al.*, 1954) ; a stimulation of pituitary gonadotrophic activity by cortisone has been considered responsible for such changes. In view of the author's previous findings on the adrenal cortex, it seemed to be of interest to examine whether 19 NT antagonizes the stimulatory action of cortisone on the ovary, if any, in sexually immature rats. Unfortunately, the results obtained by the author are disappointing as in the first place no evidence whatsoever of a stimulating effect of cortisone on the ovary is obtained. There are no ponderal, histologic or metabolic alterations in the ovary after cortisone treatment. In the second place, simultaneous administration of cortisone and 19 NT fails to produce any change in the ovary of majority of the animals, though indications of ovarian stimulation are noticeable in some. The latter could not be ascribed to any specific effect of cortisone as similar response of the ovary is observed in some animals injected with the same dose of 19 NT alone. It should be mentioned in this connection that Moore (1953) also failed to observe any effect of cortisone on the ovary of rats.

Blivaiss *et al.* (1954) reported that the increase in ovarian weight after cortisone treatment tends to regress on withdrawal of the drug. The similar procedure in the present study causes a depression of absolute weight of the ovary, but not of the relative weight. Such inhibition of the absolute weight is, however, unaccompanied by any histologic or metabolic alterations in the organ. That any residual catabolic effect of cortisone on body weight plays no part in the commensurate loss of ovarian weight is clear from the fact that the body weight recovers on cessation of cortisone treatment and becomes almost at par with that of the controls (Table I, *Experiments IV and V*). Whether the ovary after an initial refractory phase during the administration of cortisone, tends to lose weight in the post-treatment period cannot be inferred from the present data. Nevertheless, it is interesting that cortisone pre-treatment does not seem to interfere with the action of 19 NT on the ovary except that the cholesterol content tends to show a drop. This might indicate a conditioning effect of cortisone on the synthetic apparatus of the ovary but again the meagre data at hand would make such a remark unjustified.

Effect of 19 NT on the uterus

The effect of androgens on the uterus in general, simulates that of progesterone (Dorfman and Shipley, 1956). In spayed rats the shrinkage in weight of the uterus which normally attends ovariectomy is reduced or prevented by TP (Callow and Parkes, 1935 : Aschheim and Varangot, 1939 ; Korenchevsky and Hall, 1940 ; and others). The endometrium becomes greatly thickened and develops lace-like foldings resembling the endometrium of pregnancy or that seen after combined treatment with oestrogen and progesterone. Similar changes have been reported in the uterus of intact rats and also in the same organ of other species (Korenchevsky and Hall, 1940 ; and Dorfman and Shipley, 1956). The progestional action of androgens is further testified by the fact that TP causes mucification rather than cornification of the vaginal mucosa in ovariectomized rats (Dorfman and Shipley, 1956).

It is interesting that marked progestional effects are shown on the uterus by some chemical relatives of testosterone like ethinyltestosterone, 19 NT, 17 α-methyl-19 NT, 17 α-ethyl-19 NT, and 17 α-ethinyl-5(10)-oestraenolone (Dorfman and Shipley, 1956; Ferin, 1956; and Pincus *et al.*, 1956*a*). Furthermore, 19 NT has been reported to possess anti-oestrogenic property (Ferin, 1956; and Payne *et al.*, 1956) which, again, points towards a progesterone-like action. It is, however, not known whether the progestional effects of these compounds bear any relationship to the extent of their androgenic properties.

19 NT administration to immature rats both intact and ovariectomized, causes significant increase in the absolute and relative uterine weights accompanied by marked oedema and hyperaemia. The proliferative and glandular changes in the endometrium are typically progestional in the higher dosage groups (1.5 and 3 mg.). This compound also mobilizes glycogen and alkaline phosphatase in the uterus and these are particularly noticeable in the higher dosage groups. It is interesting that progesterone can induce similar deposition of glycogen in the uterus (Burrows, 1949). Moreover, like progesterone, 19 NT causes mucification but not full cornification of the vaginal mucosa of intact and ovariectomized rats. The bulk of evidences, therefore, point towards the progestional property of 19 NT on the uterus and tends to confirm similar findings of Ferin (1956) on the human endometrium and Saunders *et al.* (1957), on the endometrium of rabbits.

19 NT pre-treatment interferes with the response of the uterus to serum gonadotrophin in a sense without producing any untoward effects on the organ. It is a commonplace that in the immature animals exogenous gonadotrophic hormone stimulates the uterus *via* the ovary; the histophysiologic consequences of such stimulation are actually ascribable to the precocious production of ovarian hormones under the influence of gonadotrophin. It is, therefore, interesting that the histologic and metabolic features of the uterus in the group which received serum gonadotrophin subsequent to 19 NT are similar to those of the group injected with the steroid alone. The nature of proliferative changes in the endometrium of animals treated only with serum gonadotrophin indicates the production of progesterone by the ovary. However, contrary to one's expectation there is no summation effect of the endogenously produced progesterone on the uterus already stimulated by 19 NT. Perhaps the uterus responds maximally to 19 NT (at the dosage used in this experiment) and as such becomes refractory to any further progestional stimulation. Whether progesterone produced by the ovary under the influence of serum gonadotrophin tends merely to maintain the proliferative conditions in the uterus already induced by 19 NT could not be deduced from the data at hand.

Cortisone treatment fails to evoke any response in the uterus except a loss in its absolute weight. The relative weight and other features of the organ, however, remain unaltered. These findings again, are at variance with those of Blivaiss *et al.* (1954) who reported an increase in uterine weight after cortisone treatment, though they did not find any significant histologic changes in the organ. Moore's (1953) negative findings might also be recalled in this connection. It is interesting that cortisone does not interfere with the action of 19 NT on the uterus when the two are administered simultaneously and in this respect the similarity with the ovary is striking.

Administration of 19 NT subsequent to cortisone treatment produces typical progestional changes in the uterus. There is no indication of interference with the action of 19 NT due to prior treatment with cortisone. In the group which received cortisone alone there is only a loss in the absolute uterine weight in the post-treatment period, but otherwise the organ appears virtually normal. Here again, the overall pattern of response seems to be similar to that of the ovary.

Effect of 19 NT on pregnancy

The suppression of oestrus during TP treatment has been repeatedly observed in rats and mice, but the effect is transitory in the sense that the cyclicity of the phenomenon resumes within a few days after the cessation of injections (Burrows, 1949). Similar effects of TP on the menstrual cycle in Rhesus monkeys have been recorded by Hartman (1937) and Zuckerman (1937). Slechta *et al.* (1954), reported that ethinyl testosterone inhibits ovulation and oestrus in rats without affecting pregnancy. Recently, a number of chemical relatives of testosterone like 17 α-ethinyl-19 NT, 17 α-ethinyl-5(10)-oestraenolone and 17 α-ethyl-19 NT have been shown to possess conception inhibiting activity in rats when administered orally (Pincus *et al.*, 1956*b*). Of these, 17 α-ethinyl-5(10)-oestraenolone has been noted to be most active. The recipient female rats do not conceive in spite of the fact that they mate otherwise successfully. It is interesting that this compound and 17 α-ethinyl-19 NT tend to disturb the menstrual cycle in the human (Rock *et al.*, 1956).

The present series of experiments reveal some noteworthy facts regarding the influence of 19 NT on mating and pregnancy in rats, although the *overall pattern* of effects on these phenomena seems to be similar to that exhibited by some of its chemical relatives (see above). Thus, it is clear that this compound is also capable of exerting some effects on mating and pregnancy provided it is *injected* during the mating period. On the other hand, if such treatments are restricted to the pre-mating stage then only a transient irregularity of the oestrus cycle is produced which tends to disappear during the post-treatment period ; and no effect on mating or pregnancy is observed. *Oral administration* has no influence on mating or pregnancy. Further, the present data suggest that to inhibit pregnancy it becomes necessary to inject 19 NT *continuously* for more than 24 days beginning from the very first day of cohabitation. Short-term treatments for 24 days at the same dosage level causes a significant delay in mating, but do not stop pregnancy. Whether a higher dosage level but less frequent injections will be equally effective in inhibiting pregnancy cannot be predicted from the present data.

In general, two consistent effects of injection of 19 NT to adult female rats are a characteristic irregularity of the oestrus cycle and delay in mating. The irregularity of the oestrus almost invariably consists of the appearance of mixed type of cells in the vaginal smears during oestrus instead of cent percent cornified cells characteristic of full oestrus. It should, however, be noted that although the oestrus stage is not clearly defined in the vaginal smears of 19 NT treated animals yet the cyclicity of the oestrus phenomena remains virtually undisturbed. Such irregularity perhaps indicates a partial suppression of oestrus which is borne out by the fact that mating occurs in 42 out of 46 injected animals (Table II) in spite of the presence of this irregularity at one time or another during treatment. Further, the acceptance of male by the female rat only at oestrus (Slechta *et al.*, 1954) adds verisimilitude to such a supposition. It is also interesting to note that similar mixed type of cells instead of exclusively cornified ones are noticed in the vaginal smears of sexually immature rats injected with 19 NT during the pre-pubertal stage (see also *Experiments I and II* under section B.).

As already mentioned the delay in mating occurs in those animals which are injected with 19 NT only during the mating period. In short-term treatments (for 24 days) all of such matings prove fertile if and when they occur. The delay in mating due to short-term treatments also suggests a delay in ovulation, but not its complete suppression. If the progesterone-like properties of 19 NT are taken into consideration such an effect on ovulation is not surprising. In point of fact, Slechta *et al.* (1954) have actually recorded such effects of progesterone on mating and ovulation in rats.

In contrast to the delay effect of short-term courses of 19 NT on mating and probably on ovulation, the long-term injections either delays or completely suppresses mating. Moreover, as pointed out before, such matings prove sterile as not a single pregnancy occurs throughout the treatment period ; the animals eventually pass into a persistent dioestrus condition characteristic of pregnancy in normal animals. These findings suggest that in the animals which do not mate at all due to prolonged injections the oestrus and ovulation are completely suppressed and not merely delayed as in the short-term groups.

The occurrence of sterile matings in animals subjected to long-term treatments with 19 NT seems curious. This cannot be be explained solely on the basis of the suggested delaying effect of this compound on ovulation, because in other groups treated similarly during mating successful pregnancy invariably occurs. Moreover, a perusal of Table II (column 3) will clearly indicate that the extent of delay in mating is not much different in various experimental groups (*Experiments II to IV*, Table II). The suggested delay in ovulation may also be taken to be within comparable limits in the different experimental groups. Tho absence of any overt indications of abortion adds further to the confusion. It may be that in addition to its delaying effect on ovulation 19 NT inhibits fertilization and a combination of these two effects are perhaps responsible for the suppression of pregnancy. It is interesting that Pincus *et al.* (1956*b*) have made similar suggestions regarding 17 α-ethinyl-5(10)-oestraenolone. Reference may also be made to the finding that progesterone can inhibit fertilization in artificially ovulated ewes (Dutt and Casida, 1948). Nevertheless, such arguments do not shed any light as to why in the groups given short-term courses of 19 NT only the ovulation is delayed, but fertilization is not interferred with. The obvious pregnancy record of these animals (Table II) bears out the latter supposition. These tend to imply that *in some* 19 NT only delays ovulation, while *in others* it influences both ovulation and fertilization. Such differential actions even if corroborated by future experiments, will not defer the logical query as to the factor(s) that will determine the *nature* of effects of 19 NT on the female reproductive processes in a particular situation. This is an intriguing question indeed to answer at present, but we have an impression that the stage of the oestrus cycle at which the treatment originally starts might be one of the factors responsible for determining the ultimate nature of effects to be exerted by 19 NT. Unfortunately, we have no data to substantiate this hypothesis, as in the present experiments no attention has been paid to this particular point at the time of commencement of 19 NT treatments. Nevertheless, it may be interesting to note that in the ewes FSH injections during the anoestrus phase have no effect on fertility, but if the same treatment is given during oestrus there is improvement of fertility (Murphree *et al.*, 1944).

The conception-inhibiting effects of 19 NT are evanascent as following the cessation of injections the oestrus cycle is resumed in the normal manner even though after an appreciably long sterile period. The animals mate successfully and give birth to normal young ones. These indicate that 19 NT does not permanently alter the basic endocrine mechanisms controlling the various steps of the reproductive cycle in the female rat. Further, the absence of any untoward effects on sexual maturity and fertility in female rats injected with this compound during the pre-pubertal stage (Table I, *Experiment I*) provides additional evidence to such a view-point. It is interesting that 17 α-ethinyl-5(10)-oestraenolone has been reported to have similar temporary effects on mating and conception in rats (Pincus *et al.*, 1956).

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F. Summary

1. Subcutaneous injection of 19-nortestosterone in graded doses (1 to 3 mg.) to sexually immature female rats evokes no significant changes in the weight of the ovary or in its total cholesterol content. In general, no consistent histologic changes are produced by 19 NT in the ovary, but in higher dosage levels follicular muturation and corpus luteum formation are noticed in some animals. The concentration of alkaline phosphatase in the ovary rises in 1 and 1.5 mg. dosage groups, but drops below the control level in the 3 mg. group.

2. 19 NT has uterotrophic properties in intact immature and ovariectomized rats. It also causes premature opening of the vagina. The macroscopic changes induced in the uterus consist of marked oedema and hyperaemia. The histological changes are progestional in nature and include proliferative transformation of the endometrium, the development and secretory activity of the glands and pronounced growth of the myometrium. There is a mobilization of alkaline phosphatase and glycogen in the uterus.

3. 19 NT has no effect on the reactivity of the ovary and the uterus of sexually immature rats to gonadotrophic hormone.

4. Cortisone fails to produce any significant change in the ovary and the uterus of sexually immature rats either *per se* or when given in combination with 19 NT. The latter evokes typical uterotrophic responses in the uterus irrespective of the manner in which it is administered with cortisone.

5. 19 NT injections to sexually immature female rats have no effect on puberty or pregnancy.

6. In mature female rats 19 NT injections invariably interfere with the oestrus cycle. Short-term treatments have no effect on pregnancy, but long-term treatments cause a complete suppression of pregnancy. The matings during such prolonged treatments prove sterile and a pseudopregnancy condition persists throughout the second month of treatment period. The mechanism of such actions of 19 NT is discussed.

7. The effects of 19 NT on oestrus and pregnancy are temporary.

8. Oral administration of 19 NT to sexually mature rats has no effect on oestrus and pregnancy.

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Explanation of Plates

(All figures are photomicrographs of tissue sections. Figs. 1-4, 11, 12, 16 and 17 are magnified ×180; figs. 6–10, 13–15 and 18 are magnified ×120).

Plate V

Fig. 1. Ovary of a control rat. Note immature condition. H and E.
Fig. 2. Ovary of a rat injected with 1.5 mg. of 19 NT daily/10 days. Note the presence of a corpus luteum and growing follicles. H and E.
Fig. 3. Ovary of a control animal. Note marked phosphatase activity in the granulosum of growing follicles and in the interstitium. The presumptive theca region of a more advanced follicle shows intense staining reaction. Gomori technique.

Fig. 4. Ovary of a rat injected with 3 mg. of 19 NT daily/10 days. Note weak phoshhatase activity in the interstitium and in the granulosum. The sinusoids of the corpus luteum retain considerable amounts of the enzyme. Gomori technique.

Fig. 5. Uterus of a control rat. Note infantile condition. H. & E.

Fig. 6. Uterus of a rat injected with 3 mg. 19 NT daily/10 days. Note the proliferative changes in the endometrium which are typically progestional in nature, growth of the muscularis and general oedema condition. H. & E.

Fig. 7. Uterus of a control rat. Note the distribution of alkaline phosphatase. Gomori technique.

Fig. 8. Uterus of a rat injected with 3 mg. of 19 NT daily/10 days. Note pronounced phosphatase activity in the endometrium. An endometrial cyst is present. Gomori technique.

Fig. 9. Uterus of a control rat. Very little glycogen is present. PAS reaction.

PLATE VI

Fig. 10. Uterus of a rat injected with 3 mg. of 19 NT daily/10 days. Note the deposition of glycogen in the endometrium. PAS reaction.

Fig. 11. Ovary of a rat injected with serum gonadotrophin. Note the hypertrophy of the interstitium, bulky luteinization and a haemorrhagic follicle. H. & E.

Fig. 12. Ovary of a rat injected with serum gonadotrophin. Note marked phosphatase activity. Gomori technique.

Fig. 13. Uterus of a rat injected with serum gonadotrophin. Compare with fig. 6. H. & E.

Fig. 14 Uterus of a rat injected with serum gonadotrophin. Note marked phosphatase activity. Gomori technique.

Fig. 15. Uterus of a rat injected with serum gonadotrophin. Note the distribution of glycogen in the endometrium. PAS reaction.

Fig. 16. Ovary of a rat injected conjointly with cortisone and 19 NT/10 days. Note the presence of corpus luteum and more advanced stage of follicular growth. H. & E.

Fig. 17. Ovary of a rat injected conjointly with cortisone and 19 NT/10 days. Note the distribution of alkaline phosphatase. Compare with fig. 3. Gomori technique.

Fig. 18. Uterus of a rat pre-treated with cortisone and subsequently injected with 19 NT/10 days. Note marked phosphatase activity in the endometrium. Gomori technique.

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THE EXTENSION OF LARVAL DURATION IN *TROGODERMA GRANARIUM*, EVERTS AFTER DDT TREATMENT

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(Communicated by M. L. Bhatia, F.N.I.)

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INTRODUCTION

Practically no record is now available of the action of insecticides on the subsequent development of insects treated whilst immature. Ferguson (1942) observed a longer period of larval development when the larvae of *Prodenia eridania*, (Cram.) were reared on foliage treated with sublethal doses of basic copper arsenate and acid lead arsenate. Wene (1947) reported a retarded development in *Epilachna varivestris*, Muls., and *Pieris rapae*, L. after the application of a sublethal dose of cryolite. The extension of larval period after sublethal treatment with DDT, BHC and pyrethrum has been already recorded in *Trogoderma versicolor*, Creutz. (Shantaram, 1954).

The two species of *Trogoderma*, namely, *T. versicolor* and *T. granarium*, have been observed to show similar reactions to the oil treatments as far as their fertility and fecundity are concerned (Shantaram, 1957). A study of the responses of *Trogoderma granarium* to various insecticidal treatments must be helpful to compare the behaviour of these two species towards various treatments. This paper covers the first of a series of experiments to investigate the effects of sublethal DDT treatment on *T. granarium* and deals only with the extension of larval duration after the treatment.

MATERIAL AND METHODS

Trogoderma granarium, Everts. ("Khapra beetle") was reared at 36°C (±1°C) and 50 per cent R.H. as far as possible under optimum conditions (Hadaway, 1945), on a diet of wholemeal flour of wheat supplemented with 5 per cent dried brewer's yeast.

Mature larvae, all drawn from one generation, were selected for treatment, with DDT dissolved in oil, and were allowed to crawl on impregnated filter papers. The solutions of DDT in BOC White Oil (a Burmah-Shell product) were diluted with four times their volume of 'Basynth' petroleum ether (B.R. 80–100°C) to provide even distribution over the 7 cm. No. 1 Whatmann filter papers used. The concentrations of the insecticide employed were 1.0 per cent, 2.0 per cent and 4.0 per cent; and 0.5 ml. of the mixture was pipetted on each filter paper so as to give the standard rate of 0.0021 ml. per sq. cm. The volatile petroleum ether plays no further part in the test once it has ensured the even spread of the oil. It was allowed to evaporate before the larvae were permitted to crawl on the treated filter papers. Sets of control were run on filter papers treated with BOC White Oil and with petroleum ether alone, the latter for assessing the rôle of the oil, if any, in producing the effect. The larvae were treated for 24 hours, after which period they were kept in 1 lb. jars, on a normal diet at the same artificial conditions of temperature and relative humidity as those of the original stocks. These jars were examined every day and the pupae were separated into 2×½ inch tubes, one in each tube, where they were kept for adult emergence. This method

makes it possible to note the exact time taken by the larva after the treatment to pupate and the pupal duration of each individual.

The food consumption of treated larvae was obtained by direct weighing of the tubes in which larvae were confined after treatment, with precautions against the undue absorption of atmospheric moisture or the accumulation of faeces or of exuviae.

Results

Table I shows the number of larvae and pupae dying prior to adult emergence. Oil treatment causes a significant ($P < 0.001$) mortality in larval as well as pupal stages, but even 4.0 per cent DDT solution failed to kill more larvae than died after plain oil treatment.

Table I

Mortality in Trogoderma granarium *after DDT treatment*

Treatment	No. of Larvae treated	Larval Mortality	Pupal Mortality	Total Mortality	Percentage Mortality
Control (Oil treatment)	500	70	16	86	17.2
1% DDT	500	82	11	93	18.6
2% DDT	500	75	14	89	17.8
4% DDT	500	86	13	99	19.8
Untreated	500	30	8	38	7.6

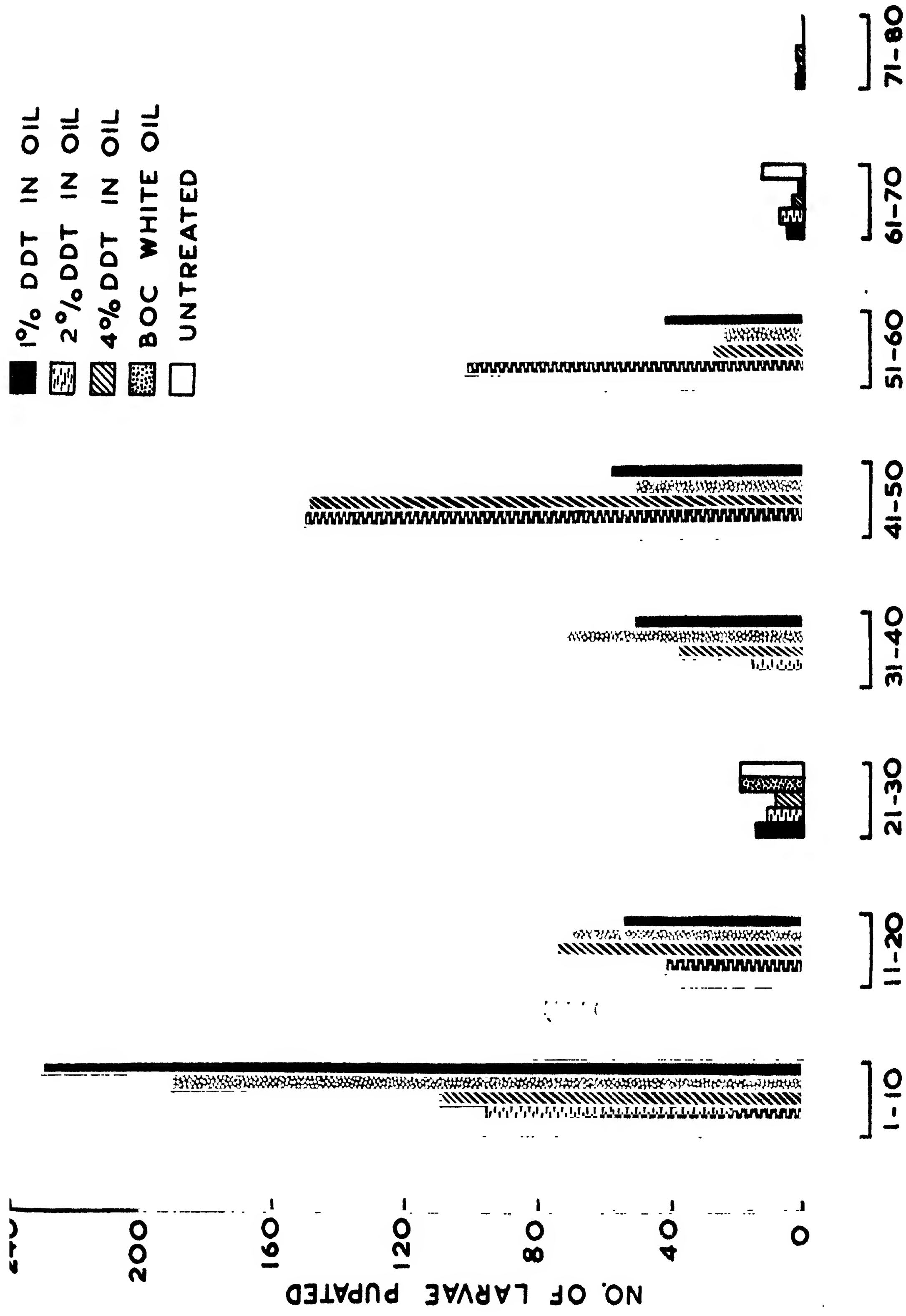
A large uniform batch of larvae which had already passed through three moults was selected for treatment. Such mature larvae should pupate within about 10–15 days (Hadaway, 1945). A few individuals normally have a greatly extended larval period up to 70 days beyond the third moult. All those larvae which pupated within 20 days of treatment were classified as 'early-pupating' and the rest as 'late-pupating'.

Table II and the graph show the larval duration of treated as well as untreated larvae while Table III shows the number of larvae in each category after treatment and the sex ratio of the emergent adults. The insecticidal treatment manifestly alters the proportion of early and late pupating larvae, in what is, in fact, a clearly bimodal distribution.

Table II

Larval duration of the mature larvae of Trogoderma granarium *after DDT treatment*

Treatment	No. of larvae per the duration of larval period in days								Total
	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	
Control (Oil)	190	71	20	72	51	25	1	–	430
1% DDT	128	37	15	18	139	74	5	2	418
2% DDT	95	42	12	15	150	102	8	1	425
4% DDT	109	75	9	38	149	28	4	2	414
Untreated	230	55	20	51	58	43	13	–	470


1% DDT IN OIL
2% DDT IN OIL
4% DDT IN OIL
BOC WHITE OIL
UNTREATED
NO. OF LARVAE PUPATED
0
40
80
120
160
200
1-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80

The data in Table III were analysed to test the significance of the increased proportion of treated larvae pupating late. The oil treatment was found to produce no appreciable effect, whereas $\chi^2_{(3)}$ for DDT is 75.8836, which value significantly exceeds expectation, showing that DDT treatment retards the development of the larvae considerably. Further χ^2 tests were carried out on individual treatments in order to find any significant difference in the proportion of sexes emerged from the two batches of larvae. There was no significant difference in the proportion of sexes in all the treatments, indicating that the prolonged larval development does not upset the proportion of sexes in the two batches.

TABLE III

Time of pupation of the mature larvae of Trogoderma granarium, *after DDT treatment and the sex of the adults emerged from them*

Treatment	Early Pupation: No. of Larvae Pupated	Early Pupation: Pupal Mortality	Early Pupation: Male	Early Pupation: Female	Late Pupation: No. of Larvae Pupated	Late Pupation: Pupal Mortality	Late Pupation: Male	Late Pupation: Female
Control (Oil)	261	12	48	201	169	4	35	130
1% DDT	165	6	39	120	253	5	51	197
2% DDT	137	8	12	117	288	6	22	260
4% DDT	184	5	18	161	230	8	17	205
Untreated	285	8	76	201	185		49	136

Table IV shows the amount of food consumed by the mature larvae after DDT and oil treatment over a period of 7 weeks. Analysis of the data indicates that the larvae treated with the oil and DDT consume less food than the untreated larvae do.

TABLE IV

Food consumption (in milligrams) by the mature larvae of Trogoderma granarium *after DDT and oil treatment over a period of 7 weeks*

Serial No. of the larva	Oil	1% DDT	2% DDT	4% DDT	Untreated
1	20.8	18.1	14.9	15.6	36.2
2	56.9	51.0	49.1	31.6	62.8
3	49.3	43.3	42.5	51.7	54.9
4	33.5	11.6	16.5	29.3	51.3
5	50.7	37.0	38.3	60.1	64.7
6	29.7	30.6	52.6	29.4	41.6
7	36.2	35.8	76.5	15.9	83.4
8	41.6	26.3	24.3	28.6	69.9
9	50.5	45.3	19.7	15.9	34.1
10	41.6	26.3	44.9	24.8	29.8
11	39.2	47.7	50.1	39.7	35.6
12	50.8	32.8	47.3	21.6	76.4

Table V shows the pupal duration of *Trogoderma granarium* receiving treatment whilst immature. The mean of the pupal duration falls between 3 and 4 days in all cases whether the larvae were treated or not. The treatments have no appreciable effect on the pupal duration.

TABLE V

Pupal duration of Trogoderma granarium *after DDT treatment*

Treatment	No. of pupae per the duration of pupal period in days						Total
	2	3	4	5	6	7	
Control (Oil)	2	118	150	89	54	1	414
1% DDT	1	110	203	63	30	–	407
2% DDT	8	131	153	91	23	5	411
4% DDT	5	128	157	79	26	6	401
Untreated	13	118	189	90	48	4	462

A study was also made of the length of the adult life. The newly emerged adults were kept under observation in $2 \times \frac{1}{2}$ inch sample tubes, one in each tube, until they died. No significant difference caused by the treatments was seen in the length of the adult life. The observations on adults from treated larvae were indistinguishable from those on untreated larvae. The female lives from 5–11 days the average being 9 days. The male outlives a fertilised female by a day or two. But a female which has failed to oviposit, even after pairing with a normal male, outlives the normal male by a considerable length of time, the highest figure recorded being 21 days.

Experiments were conducted to find out the number of moults undergone by the larvae after treatment. The average number of moults in all cases was 7 and the treatments did not alter the total number of moults significantly ; but the early-pupating and the late-pupating larvae did show a difference in the interval between successive moults. In the former case this interval was always constant the larvae moulting at a regular interval of 5–6 days, while in the latter, the interval between the successive moults was irregular, occasionally extending to as long as 14 days.

DISCUSSION

The present work discloses that the normal growth of the larvae of *Trogoderma granarium* is disturbed by DDT treatment. Factors known to extend the larval duration in an insect are temperature and relative humidity, nutritive value of the food (Hadaway, 1945) and of the parental food (Reynolds, 1945) starvation (Wodsedalek, 1912, 1917) and the density of insect population in the culture medium (Park, 1938). Care was taken to provide uniformly optimum conditions of development for the larvae in the present experiments. Larvae were drawn from one generation with no difference in the parental diet. Precautions were taken not to crowd the larvae.

Though ample food was supplied to all the larvae, there was still a possibility that the treatments could so paralyse the treated larvae as to induce partial starvation. The food consumption of the larvae treated with the oil and DDT was definitely less than that of the untreated larvae. Though the larval duration

of DDT-treated larvae was extended, the deficiency in food consumption did not cause any appreciable difference in the larval duration of the oil-treated larvae. Hence, food consumption seems to be of little consequence in determining the rate of development in *Trogoderma*.

The part played by the nervous system in the growth of an insect is well known. The function of the corpus allatum in controlling metamorphosis, by causing the insect to retain its larval or juvenile characters when it moults, has been confirmed in most groups of insects.

In the present series of experiments the retarded development is significant in the DDT-treated larvae, not in the oil-treated ones. DDT is a well known nerve poison and it seems reasonable to assume that it affects the nervous system and thereby upsets the usual growth. Wigglesworth (1955) discusses two instances where DDT has a putative action in so disturbing the nervous system that abnormal metamorphosis occurs.

In the retarded development of the larvae are involved two distinct phenomena : (i) the larval moults observed up to pupation, and (ii) the postponement of pupation. These two processes are separate and are regulated by two different hormones (Wigglesworth, 1954). DDT treatment did not alter the total number of moults ; but the early-pupating and the late-pupating larvae showed a difference in the interval between the successive moults. Wigglesworth (1934) has already shown that the moulting in a larva begins when the moulting hormone reaches its operative concentration in the blood. Hence, it seems appropriate to assume that DDT, being a nerve poison, obstructs but does not stop the regular secretion of this hormone. The rate of secretion may be reduced so that a much longer time than the normal is taken by the hormone to reach the required concentration to induce moulting. If this hypothesis is accepted, it explains why the moulting takes place irregularly in the late-pupating larvae.

For the postponement of pupation, the formation of a larval cuticle, instead of an imaginal one, at each moult is necessary. This formation is possible only in the presence of a juvenile hormone secreted by the corpus allatum. In view of the postponement of pupation observed in the late-pupating larvae, it may be suggested that the insecticidal treatment in these larvae, acting through the nervous system, induces the corpus allatum to secrete the juvenile hormone longer than required. This effect appears to fade away gradually as the larva extends its larval life and eventually pupation occurs. The irregularity in the interval between the successive moults is maintained until pupation, which indicates that the decreased rate of hormone secretion continues up to the end. In the larvae with retarded development no irregularities were observed either at the ordinary larval moult or at the pupal moult. Once the moulting is induced, the epidermal cells complete the process as if they are normal. Hence. the epidermis with the oenocytes and the dermal glands appears to be least affected in the late-pupating larvae.

Further, it is clear now that in responding to DDT treatment by retarded larval development, *Trogoderma granarium* resembles very closely its allied species, *T. versicolor*.

Acknowledgements

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Summary

A prolonged larval duration is observed in a proportion of the larvae of *Trogoderm granarium*, Everts, surviving DDT treatment. The proportion of 'late-pupating' larva

is higher in the insecticide-treated batches than in the oil-treated or in the untreated batches. No effect of DDT is observed on the pupal duration or on the duration of adult life of the survivors.

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Issued January 28. 1958.

COMPARATIVE MORPHOLOGY AND ONTOGENY OF FOLIAR SCLEREIDS IN SEED PLANTS

II. *LINOCIERA* SWARTZ

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(Communicated by P. Maheshwari, F.N.I.)

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Sclereids are distributed in various parts of the plant body including the floral parts (Morley, 1953 ; Foster, 1955*a*, *b*). In spite of their reported occurrence in the bark, pith, cortex, phloem, petiole and leaves, not many attempts have been made to make a comparative study of the range of form, and of the ontogeny of the sclereids in a single species or in several species belonging to one genus. Only Tschirch (1889) and Francken (1890) did some work in this direction. Recent studies by Foster (1946, 1955*b*) and Rao (1951*a*, 1953, 1957) have emphasised the constancy of sclereids in the leaves of many seed plants as an intersting factor in taxonomic analysis. Rao (1950) gave a detailed account of the types and distribution of sclereids in the leaves of six species of *Linociera*. Further comparative studies were made to elucidate the sclereid topography and ontogeny of certain species of *Linociera*, an account of which is presented in this paper.

Methods and Materials

The technique employed is the same as outlined in the previous paper (Rao, 1957).

The materials for this investigation are derived from fresh collections of leaves collected from different places, as noted below. Specimens were obtained from several Botanic Gardens, through the courtesy of their curators. Fixed material of species of *Linociera* for ontogenetic studies were obtained from the Curator, Royal Botanic Garden, Bogor, Indonesia.

The following fresh specimens of *Linociera* were examined :—

1. *L. caudata* Coll and Hemsl. Royal Botanic Gardens, Bogor, Indonesia.
2. *L. courtallensis* Bourd. Kemangundi, Mysore State Forests, India.
3. *L. elliptica* Knobl. Royal Botanic Gardens, Bogor, Indonesia.
4. *L. insignis* C.B. Clarke. Royal Botanic Gardens, Bogor, Indonesia.
5. *L. intermedia* Wight. Coimbatore, Madras State, India.
6. *L. lancifolia* Ridley. Royal Botanic Gardens, Bogor, Indonesia.
7. *L. macrophylla* Wall. Assam, India.
8. *L. malabarica* Wall. Kemangundi, Mysore State Forests, India.
9. *L. pauciflora* C.B. Clarke. Royal Botanic Gardens, Bogor, Indonesia.
10. *L. ramiflora* Wall. Royal Botanic Gardens, Bogor, Indonesia.
11. *L. rostrata* F. & B. Royal Botanic Gardens, Bogor, Indonesia.
12. *L. terniflora* Wall. Royal Botanic Gardens, Bogor, Indonesia.
13. *L. wightii* Clarke. Kemangundi, Mysore State Forests, India.
14. *L. zeylanica* Gamble. Indian Botanic Gardens, Sibpur, Calcutta, India.

Sclereid types

The present survey of 14 species revealed the presence of sclereids of various forms. They are segregated under three types :—

Type I :

Filiform sclereids are encountered in the following species :—

L. caudata, *L. elliptica*, *L. courtallensis*, *L. lancifolia*, *L. malabarica*, *L. pauciflora*, *L. rostrata*, *L. terniflora*, *L. wightii* and *L. zeylanica*.

The sclereids are very much drawn out and run in a criss-cross manner inside the lamina. They form a regular strand at the margins. The mature sclereids have thick striated walls and narrow lumina of uniform width.

Based on the mode of branching, three form-variations can be distinguished within this type :—

(1) The sclereids are unbranched with fusoid ends. Such cell forms are seen in *L. courtallensis*, *L. elliptica*, *L. rostrata*, *L. wightii*, *L. malabarica*, *L. terniflora* and *L. zeylanica*.

(2) The filiform sclereids show a tendency to fork. This is seen in *L. pauciflora* and *L. lancifolia*.

(3) The cell forms under this category constantly show a tendency to dichotomise. This type is encountered only in *L. caudata*.

Type II :

Rao (1950) gave a detailed account of short fusoid and polymorphic cell forms. Further observations are recorded on a comparative basis.

The sclereids in *L. intermedia*, *L. macrophylla* and *L. ramiflora* are remarkable for their varied disposition inside the lamina. In mature leaves, the sclereids are present in abundance near the midrib and marginal regions, whereas in the sub-marginal regions they are very sparingly seen. Repeated examination of a number of mature leaves have indicated that sclereids are mostly diffuse in distribution but occasionally one could see the proximity of sclereids at the vein ends. The sclereids are vertically disposed and rarely exhibit horizontal disposition. In transections, they are disposed in the form of idioblasts in the palisade region (Figs. 5 & 6). The idioblastic sclereids are sometimes disposed in such a way that they connect the base of peltate scales and the vascular bundles in the leaves. This feature is very often seen in *Linociera macrophylla* and rarely in *L. intermedia* and *L. ramiflora*. The sclereids do not extend and form columnar cells touching both the epidermal regions. They protrude slightly into the air-space system of the spongy region. These sclereids show a good deal of variation. In spite of this, the cell forms are mainly fusiform or osteosclereids with a tendency to produce short arrested processes. As already observed in *L. macrophylla*, the sclereids exhibit two distinct lines of cell modifications (Rao, 1950). The sclereids of *L. intermedia* are more or less fusoid with short knob-like processes all over the cell body, whereas the sclereids of *L. rostrata* do not exhibit a wide range of cell modification. They are more or less pillar-like prop-cells with short branches at the abaxial side. Irrespective of their cell forms, they have thick striated walls and lumen of irregular width. The pit canals are present and they are straight or oblique in disposition.

Type III :

The cell forms under the type are stellate or dendroid sclereids. They are abundantly present in the lamina of *L. insignis*. The cleared lamina shows the abundance of diffuse sclereids and occasionally some sclereids are found at the vein-ends. They are uniformly distributed inside the mesophyll. In transection, they form conspicuous cell forms extending into the palisade and spongy region

of the mesophyll. The sclereids possess prominent processes, which repeatedly fork giving a dendroid appearance. The sclereid cell wall is striated and the lumen is of irregular width. The cell wall has pits of varied disposition.

Ontogeny

The ontogeny of *L. intermedia*, *L. elliptica* and *L. insignis* are described in detail.

Origin and Development

The embryonic leaf tissue consists of compactly packed cells and shows very little differentiation. In the next phase the mesophyll gets differentiated into one-layered palisade cells and spongy cells with small air spaces. The sclereid initials in *L. intermedia* and *L. elliptica* are transformed palisade cells. Soon the sclereid initials exhibit a conspicuous nucleus, dense protoplasm with or without vacuoles. The initials are distributed as idioblasts and in no instance they were found *in situ*. Sometimes one could see the presence of sclereid initial cell just above the differentiating vascular bundle (Figs. 2, 4). This juxtaposition of the sclereid initial is responsible for giving the occasional presence of seemingly terminal sclereids in cleared leaves (Rao, 1951*a* ; Fig. 12). Another point of histogenetic interest in the embryonic leaves of *L. intermedia* is the transformation of a palisade cell as sclereid initial connecting the peltate scale on one end and vascular bundle at the other (Fig. 4). Unlike *L. intermedia* and *L. elliptica* the sclereid initials of *L. insignis* are transformed spongy cells (Fig. 7). This is a significant fact in view of the occurrence of sclereid initials of palisade origin in.the other two species of *Linociera*. The initiation of sclereids in the spongy region is not confined to any layer of cells. The initials are sub-spherical to polygonal in form and possess a conspicuous nucleus and dense cytoplasm (Figs. 7–10).

With the initiation of growth, the sclereid initial cell increases in its volume and protoplasm appears in strands. This is soon followed by vacuolations either at the centre or from the periphery and the nucleus migrates downwards. The growing cell in *L. intermedia* is more or less tubular and pushes its way downwards and the growth is inter-cellular. At no time the cell extension was seen touching the upper and lower epidermal layers. In the case of *L. elliptica* the tubular growing sclereid cell ramifies in the mesophyll and sometimes runs intradermally to a considerable length, whereas the growing sclereid cell in *L. insignis* is very vigorous, multidirectional and sends out processes in between adjacent cells, sometimes touching the epidermal layers. The tip of the growing cell is fusiform and the mode of growth undoubtedly gives support to the view that the apical portions of the cell plays an important rôle in separating the compactly situated spongy cells.

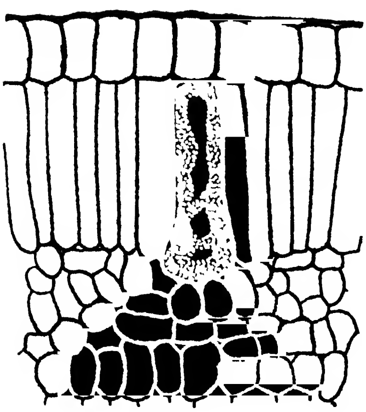
Explanation to Figures

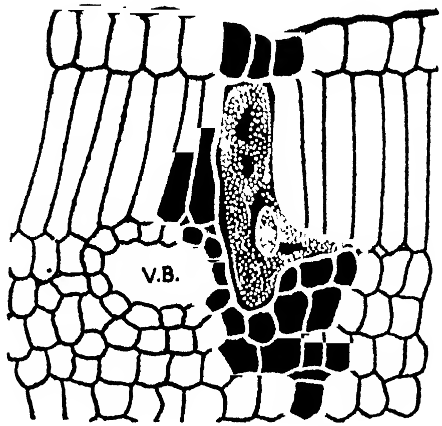
Figs. 1–6. Portions of transections of young and mature leaves of *Linociera intermedia* Wt.

Figs. 1–4. Early stages in sclereid ontogeny, ×225 each.

Fig. 1. Sclereid initial: transformed palisade cell.
Fig. 2. Sclereid initial near vascular bundle and showing bifurcation.
Fig. 3. Sclereid initial traversing horizontally.
Fig. 4. Sclereid initial connecting the peltate scale and vascular bundle.
Figs. 5–6. Vertically oriented adult sclereids showing entry of arms into air-spaces. Note thick striated wall, pit-canals, and nucleus in centre of lumen, ×225 each.

(1)





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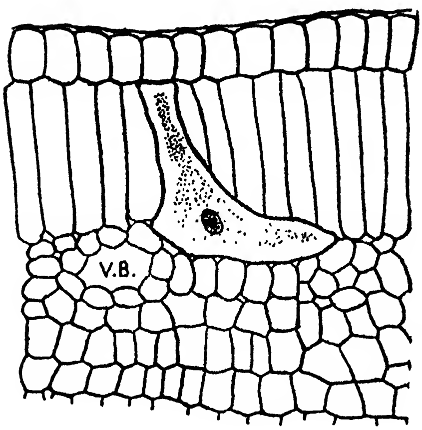

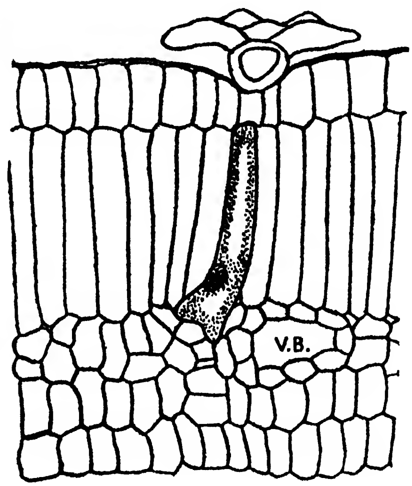

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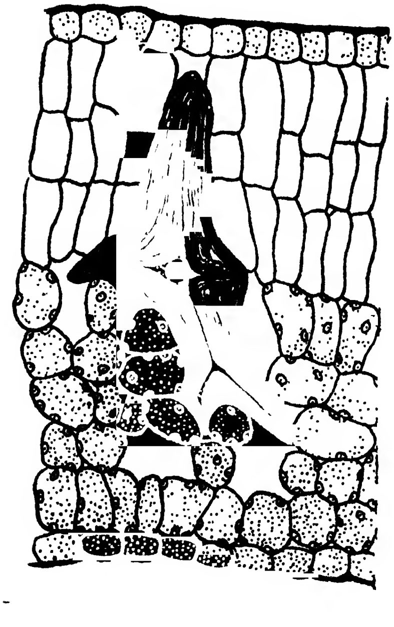

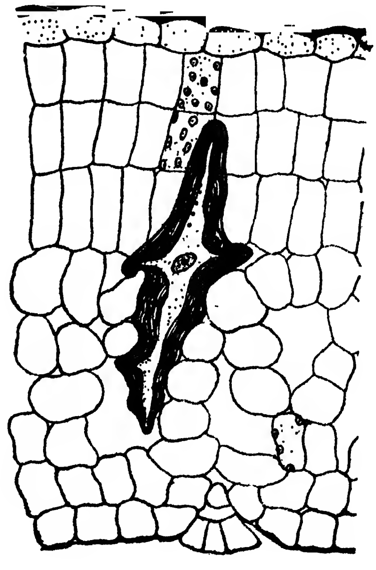

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The initiation of air-clefts and sclereids is found to extend to a considerable time in the embryonic tissue. With the initiation of growth, there is simultaneous initiation of air-spaces formation in the mesophyll, especially in spongy region. The air space system is of moderate nature, but the system of branching reveals that more branches are found in spaces where the cells are compactly disposed than in those areas where there is a regular air space formation. All initials do not seem to grow simultaneously as they exhibit different degrees of maturation. While some are in the extending stage, the other cells begin to show the process of sclerosis. The thickening is centripetal and this leads to the formation of a striated thick secondary wall. The lumen is reduced to a narrow channel or is occluded. One could see the presence of a nucleus even in such cells which have procured a massive secondary wall. (Figs. 5, 6, 11). Ultimately the nucleus degenerates and the disintegrated protoplasts are seen in the lumen region. The secondary wall shows straight or oblique pit-canals closely situated or spread over without any uniformity.

Discussion

1. *Distribution*

On the basis of their general distribution, sclereids in seed plants can be grouped under three types. Those of the first type are the *diffuse sclereids* which are dispersed in a variety of patterns in the mesophyll amidst compactly disposed cells or within the lacunate spongy parenchyma tissue. The second type includes the *terminal sclereids*, which show a terminal position at the vein ends. The third type of distribution consists in having apparently terminal as well as diffuse sclereids in one and the same lamina.

Almost all species of the genus *Linociera* possess the varied types of sclereids exhibiting a good deal of differentiation from simple prop cells to sac-like cell forms with many intermediary form types (Solereder, 1908). The present survey has revealed that the sclereids are the most important idioblasts of the lamina. Mostly, they exhibit diffuse pattern of distribution and rarely exhibit apparent terminal relationship with vein-lets. Majority of sclereids are filiform distributed in criss-cross manner inside the lamina. The next trend is the appearance of prop-like fusoid cell forms with a good deal of fluctuation in shape and size. The third type of sclereids are the astro-sclereids or profusely ramified cell-forms with radiating branches.

Recent studies by Foster (1946, 1955*b*) and Rao (1953, 1957) have emphasised the diagnostic value of sclereids in the identification of plants. In view of the constant presence of sclereids of varied types in almost all species of *Linociera*, they undoubtedly form an important factor in any taxonomic analysis.

Statements on the terminal, sub-terminal or diffuse types of distribution of sclereids are usually based on observations made on mature sclereids in the leaf

Explanation to Figures

Figs. 7–12. Transections of young, immature and mature leaves of *Linociera insignis* C.B. Clarke.

Fig. 7. Sclereid initial. Note the transformed spongy cell, ×225.

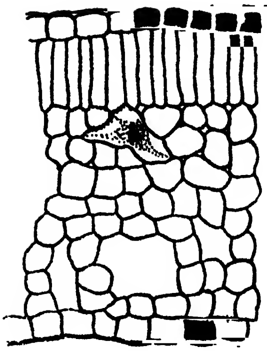
Fig. 8. Sclereid initial sending its processes into the palisade region, ×225.

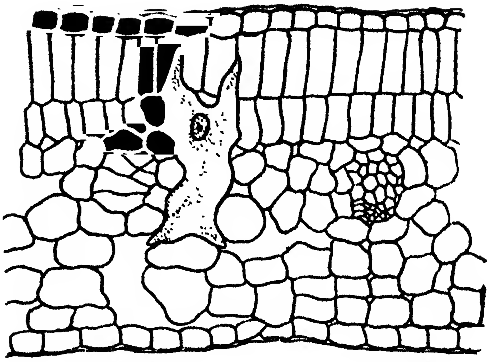
Figs. 9–10. Late stage in sclereid ontogeny. Note slightly sclerosed wall and the proximity of one of the processes near the differentiating vascular bundle in fig. 9, ×225 each.

Fig. 11. Late stage: Note thick striated wall, ×225.

Fig. 12. Adult astrosclereid: Note prominent, drawn out processes and narrow lumen and pit-canals, ×225.

(7)





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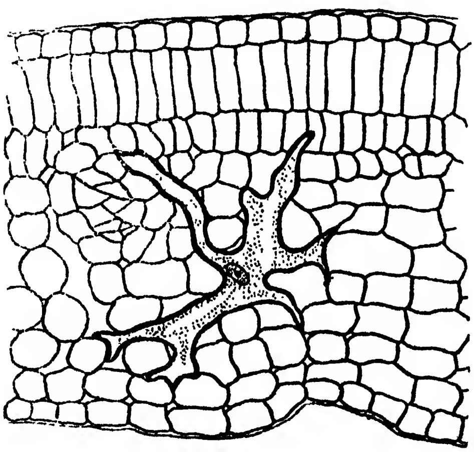

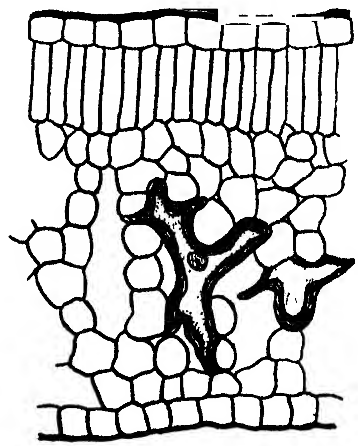

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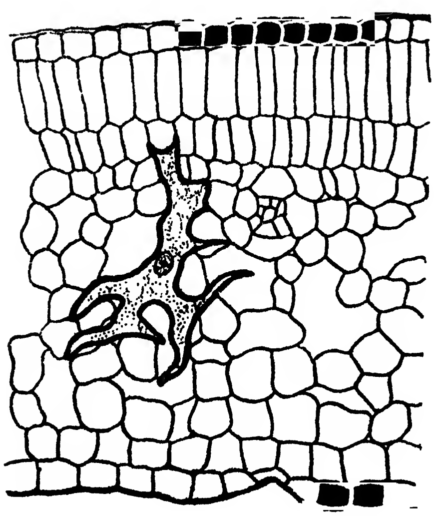

(12)

tissues but it is only in a few cases, that the early developmental stages have been studied. Recent investigations of Foster (1944, 1945, 1947, 1955*a*), Rao (1951 *a*, *b*, *c*, 1952, 1953) and Arzee (1953) have thrown some light on the major problems concerned in sclereid ontogeny.

Ontogenetic studies have demonstrated that in all the three species, the sclereid initial cell could be recognised at a very early stage of tissue differentiation in the mesophyll. It is a thin-walled enlarged uninucleate typical idioblastic cell showing dense cytoplasm. An analysis of the place of origin reveals that the sclereid initials in *L. intermedia* and *L. elliptica* are transformed palisade cells whereas in *L. insignis* they are spongy cells.

The sclereid initials are random or orderly in their disposition on the developing tissues. The diffuse sclereids do not seem to arise in the same manner or at the same time. Sometimes varied forms differing from each other in growth are encountered within the mesophyll. This clearly indicates that the initiation and development are extended over space and time. This type of sclereid initiation is reported in plants of many diverse families.

However, in certain leaves the sclereid initials are found near the ends of the procambial strand. The occurrence of sclereid initials at the terminus of the procambium is manifest in *Mouriria huberi*, *Boronia serrulata* (Foster, 1947, 1955*a*), *Memecylon heyneanum* (Rao, 1951*c*), *M. lushingtonii* (Rao, 1957), *Niebuhria apetala* and *Cynometra cauliflora* (Rao, 1953). Such sclereid initials when they grow out give the characteristic terminal or sub-terminal relationship with the vein-ends. This orderly origin of sclereids stands in contrast to the random or diffuse sclereid initials.

Ontogenetic studies on the species of *Linociera* have shown some sclereid initials near the differentiating vascular bundles and some far away from the vascular bundles in one and the same embryonic leaf. Such a type of sclereid initiation is also reported in *Diospyros discolor* (Rao, 1951*b*). Sometimes the sclereid initials which are slightly at a distance from the veins, due to their extraordinary vigorous growth, come in close contact with the veins, thereby giving the appearance of terminal or sub-terminal disposition with vein endings. A similar type of sclereid initiation is also seen in *Ternstroemia japonica* L. (Rao, 1952). In the above cases, sclereids in the mature stage indicate terminal as well as diffuse sclereids in the same leaf. Thus the present studies have emphasised the importance of ontogenetic studies before establishing the true relationship between sclereids and vein ends.

2. *The Problem of Classification*

When one studies foliar sclereids showing a great range of cell forms, differing in shape and size, the importance of analysing them into definite groups becomes apparent. In any such attempt it is impossible to avoid bringing in the importance of the concept of sclerenchyma as conceived by different authors at different times. Foster (1944, 1949) has given an admirable summary of this concept from an historical stand point. He has shown the development of two schools of thought and the usage of different descriptive terms by different authors to thick-walled cells occurring in various parts of the plant body. Much confusion exists due to the development of morphological concept from the times of Mettenius' (1865) work or due to the influence of Schwendener's (1874) physiological classification of mechanical elements.

In view of the influence of the above concepts, in any attempts to classify the thick-walled cells many difficulties arise. The main point which requires immediate attention is to find salient facts about sclereids and fibres. Sclereids, like fibres may be short or long and show pitting in greater abundance on the secondary wall than the fibre cell wall. Like fibres they often appear as idioblasts

or form regular reticulum or appear in strands. Most of the sclereids do not show any relationship with the vascular elements. However, in some cases there is intimate contact, as commonly seen in the case of fibres. Sclereids originate by the transformation of parenchymatous cells, whereas fibres originate from meristematic cells. But there are instances when sclereid initials originate at the terminus of procambial strand but there is no evidence of the conversion of procambial cell into a sclereid initial cell. Most of the sclereids are non-septate, whereas the fibres which are of common occurrence in many dicotyledons are septate. The occurrence of septate fibre-like sclereids in *Scindapsus* species (Rao, 1954) and septate brachysclereids of *Begonia Corallina* (Gertz, 1915) further emphasises the difficulty of distinguishing sclereids from fibres. Studies by Foster, (1944, 1945) and Subramanyam and Rao (1949) have revealed the existence of thin-walled cell forms of variable size and shape in leaves in which intergradations are found between the simplest spheroidal cell and the complex fibriform cells. Thus sclereids and fibres have shown certain common features. The existence of intergrading forms and also septation in the lumen does not justify the integration of the two terms into the common term. The present study supports the view expressed by Francken (1890) and Foster (1944) that fibres and sclereids in spite of their close resemblance do possess certain features which justify the retention of separate terms for them.

The term 'sclereid' to thick walled mechanical cells was introduced by Tschirch (1885, 1889). He believed that they are distinct cell forms and differ from bast fibres both in form and wall structures. In spite of the usage of such terms as spicular cells (Solereder, 1908), Sclerite (Van Tieghem, 1891), idioblasts (Sachs, 1882), trichoblasts (Sachs, 1882), stone cells (Schumann, 1889), sclereiden (Francken, 1890), sterocytes (Chodat, 1920), sclereites (Seward, 1906), the term "sclereid" is maintained because it is brief and etymologically clear and has much, therefore, to commend it (Eames and MacDaniels, 1947, pp. 88-89).

Tschirch's studies on sclereids bring to light that he was influenced by Mettenius and Schwendener and he applied the term sclereid only to such cells which are non-prosenchymatous in form. He recognised four types of sclereids according to the form of the cell :

(i) Brachysclereids which are more or less isodiametric cell forms found chiefly in pith, phloem, cortex and bark and in fleshy portions of fruits ;
(ii) macrosclereids which are columnar in form and mostly found in rows in the seed coats ;
(iii) osteosclereids which are prop-like cells with or without branching processes at the poles and seen in seeds and leaves of dicotyledons ; and finally
(iv) astrosclereids, which are cell forms exhibiting a good deal of variable form characterised in many leaves.

Thus one could see in Tschirch's studies, an attempt to segregate and identify the different types of cells occurring in different parts of the plant body. The past workers have studied the existence of different cell forms of sclereids in several species of the same genus, or several genera of the same family or order. But no attempt has been made to build up a natural classification of sclereids.

In the present analysis of sclereids occurring in several leaves the following main types of compact histological cell forms are recognised :—

(i) *Spheroidal sclereids* exhibit a good deal of form variation, from simple globoid or sub-spherical to highly lobed sac-like cell forms. They are found mainly in the abaxial part or sometimes in the adaxial part or in both parts of the midrib region. They may be terminal or diffuse sclereids.

(ii) *Osteosclereids* which are mainly disposed in the palisade region or extend to such an extent as to touch the epidermal layers. These cell forms exhibit central columnar shape with dilated short processes at the poles. They may be terminal or diffuse sclereids.

(iii) *Fusiform sclereids* have uniform width with tapering poles, with or without branching. They do not form massive columnar structure, sometimes exhibit branching at each end, especially near the epidermal layers. Just like osteosclereids they are disposed in the palisade or extend to such an extent as to touch the epidermal layers. They may be terminal or diffuse sclereids.

(iv) *Filiform sclereids* are those cell forms, which are designated as ophiurid, or fibriform cells. They are the most conspicuous features of the leaves and form a rich reticulum. They are either short, elongated or branching cell forms. They have more or less uniform width with tapering ends. They form a strand at the margins and are irregularly disposed in the sub-marginal region of the lamina. Branching cell forms form a rich intra-epidermal branching system. They may be terminal or diffuse sclereids.

(v) *Astrosclereids* are those cell forms which range from short gnarled form to regular stellate and highly branched dendroid cell forms. They are disposed in the palisade or spongy region or in the midrib region of the lamina. They may be terminal or diffuse sclereids.

(vi) *Crystalliferous sclereids* are either spherical, stellate or dendroid in cell form having distinct crystals embedded in its wall or deposited in the lumen. They may be terminal or diffuse sclereids.

The above main types of sclereids are based on form and the disposition in the mature lamina. This classification would be useful in recognising the range of cell forms of sclereids occurring in leaves. But it is defective in the sense that the origin and structure are completely ignored in building the main cell form types. This would never give us a natural system of classification.

The above mentioned form types are classified into groups on the basis of their ontogeny and their origin from epidermis, palisade and spongy cells. In building this classification considerable care is taken to observe a detailed study of early stages of sclereid development to ensure a precise method of classification. On the basis of ontogeny three main groups of sclereid development are recognised. Each group is next sub-divided into types on the basis of the main cell form in the adult condition (Rao, 1951*a*).

3. *Functions of Sclereids*

The sclereids are typical idioblastic cells distributed in various parts of the plant body. By virtue of their thick secondary wall they serve the mechanical purpose of giving additional strength and rigidity to the plant parts. Tschirch (1885) states that sclereids are concerned in producing a stiff or coriaceous texture. Francken (1890) is of opinion that the sclereids have a mechanical function in a great many cases. Haberlandt (1914) states that brachysclereids serve to increase the incompressibility of the bark, their action being compared to that of sand which a mason uses to increase the tenacity of mortar, or to that of powdered glass which is added to the gutta-percha in order to render it less compressible. According to Stevens (1924) the sclereids give mechanical toughness to the leaves against shearing stresses without being an impediment to their increase in size. Some of the above stated views suggest the importance of considering sclereids as mechanical cells.

Strasburger (1891) has expressed the view that the sclereids which occur in the bark of Larch, Spruce and Fir, and in the flesh of the pear, have no mechanical significance at all. He believes that metabolic processes which go on in the starch conducting tissues of the plants in question lead to the production of superfluous cellulose-material, which is then deposited in the form of secondary thickening layers on the walls of the sclereids. This view of Strasburger and that of Cohn's (Francken, 1890, p. 80), explanations of sclereids as useless excrements cannot, however, be accepted in view of the overwhelming evidence in support of their mechanical fulfilment in increasing the steadiness and stability of plant parts.

The past workers were chiefly influenced by the disposition and distribution of adult sclereids in the mature lamina. On the strength of the analysis of sclereids, they attributed a mechanical significance in view of the absence of a living plasma, lack of elasticity and the thick secondary wall.

First of all let us examine the distribution of sclereids as a mechanical idioblast. As we know leaves which are flat, bifacial and isobilateral structures, disposed more or less horizontally on the axis, are subjected to forces which operate perpendicular to the surface of the leaf. Generally such leaves have elongated or very much reduced petiole. Sometimes the short petiole closely adpresses to the axis as seen in *Penaea mucronata* and *Sarcocolla formosa;* by virtue of their disposition they are subjected to various mechanical pressures. To counteract against all possible pressures from outside, such leaves exhibit sclereids and the following features :—

(a) Criss-cross disposition as exemplified in most of the species of the genera *Olea*, *Linociera*. *Mouriria*, *Memecylon* showing filiform sclereids.

(b) Formation of regular strands just beneath the epidermal region as seen in the species of *Olea*, *Linociera*, *Mouriria*, *Memecylon* having filiform sclereids.

(c) Marginal disposition of circular, semi-circular or oval shaped strands composed of fusiform or gnarled shaped sclereids, more or less initimately interconnected to give a strand-like appearance. This is seen in *Maba nigrescens* of Ebenaceae (Rao and Kelkar, 1950), *Bucklandia populnea* of Hamamelidaceae (Rao, 1953) and certain members of Myrsineae (Haberlandt, 1914).

(d) Extension of sclerenchyma accompanying the vascular strands, freely into the mesophyll and showing intra-epidermal branching. This is seen in *Banksia dryandra* (Francken, 1890), *Mimusops hexandra*, *Uvaria macrophylla*, etc. (Rao, 1953).

The above mentioned disposition of sclereids undoubtedly protects the lamina from breaking and offers an ideal disposition to check against bending forces.

In contrast to the above type of distribution, one could see that sclereids in some of the leaves are disposed vertically. They are either confined to the upper half of the leaf or extend downwards to touch the epidermal layers. They are osteosclereids or fusiform sclereids. The columnar sclereids branch out at the extremities and give out root-like slender branches beneath the epidermis. Such sclereids which are confined to palisade region slightly protruding into the spongy area are in comparable position with the distribution of sclereids in soft parts of the plant body. Their presence makes the thin walled photosynthetic cells incompressible and give a good deal of protection. Ideal approach to secure maxmum protection with minimum expenditure of material is the formation of columnar sclereids touching the epidermal layers and producing branches of various types. Such a cell form contributes to a system of I-girders in the lamina thereby giving enough protection to photosynthetic and spongy cells against squeezing or shrinking. They are disposed at a distance or they are very close to each other and form more or less a connected net work by close approximation. Such cell forms are exemplified in the species of *Mouriria* and *Memecylon*. This I-girder system of the disposition of sclereids gives better protection to the underlying photosynthetic cells than osteo- or fusiform sclereids disposed in the upper half of the leaf. A very interesting situation is observed in the leaf of *Memecylon cuneatum* wherein the thick massive columnar sclereids, with or without small knob-like branches touching the epidermal layers, alternate with fusiform branched sclereids (Rao, 1957). These branches arch over the main column and act like subsidiary I-girders amidst widely separated main I-girders. A look into their disposition gives an additional proof that in such leaves every cell is well protected against external pressures.

Thus, the foliar sclereids in a majority of cases undoubtedly play a major rôle as mechanical cells. But this generalisation of sclereids as mechanical idioblasts has to be modified in the light of the following interesting features.

The discovery of terminal sclereids, pseudo-terminal sclereids, 'hybrid cells' and the bridging up of the base of peltate glands and the vascular bundles may throw some light on the physiological rôle of idioblastic sclereids. As already pointed out the terminal sclereids are observed in plants belonging to different families (Rao, 1957). There is an ontogenetic evidence to show that sclereid initials originate at the terminus of procambial cells. From a physiological point of view this orderly origin of sclereids at the terminus of procambium may be considered as 'favourable location' both for its development, growth and conduction of water. By virtue of their origin close to the procambium and in view of the fact that they are thin-walled protoplasmic contents in the early stages they may prove to be water-conducting cells to the adjacent mesophyll cells. But in later stages they may serve the chief mechanical rôle of strengthening the mesophyll. Similarly one could say on the relationship between peltate scales, sclereids and vascular bundles. In some species of *Linociera* the sclereids are distributed in such a way that they appear to connect the base of peltate scale or gland and the vascular bundle in the leaves. This interesting disposition is of great histological interest in view of the close connection of the sclereids to the peltate glands or scales which are proved to be secretory in function in *Osmanthes* (Metcalf and chalk, 1950) and vascular bundles, the sclereids being thin-walled and with protoplasmic contents in early stages they may act like a channel for the conduction of secretion and serve in later stages the rôle of the mechanical cells.

At this point it would be interesting to recall to our mind the association of sclerenchymatous cells in the form of fibres in connection with peltate, stellate, and shaggy hairs of Melastomaceae, Mimosaceae and Euphobiaceae. In such plants the bases of the hairs are formed by one or two bundles of sclerenchymatous cells, which occupy the palisade disposition. Sometimes they branch out to form an arching foot.

In the species of *Majeta*, *Myrmidone* and *Tococa* of Melastomaceae (Gottschell, 1900) the shaggy hairs are stiffened by means of isolated sclerenchymatous fibres or whole bundles of such fibres occasionally show connections with the vascular bundles in their basal portions. The significance of this is yet to be investigated.

Next to these features is the occurrence of pseudo-terminal sclereids. The exact significance of the juxtaposition of sclereid initials near the vascular bundles is, however, not yet known. It is a case of a chance occurrence or a tendency of isolated sclereids to grow as far as possible near the vascular bundle, or does it foreshadow the tendency of sclereids to arise in close connection with the procambium ?

Finally, the occurrence of 'hybrid cells' are of great significance. Though no developmental study was made on such leaves the present study has revealed that hybrid cells having characters intermediate to storage tracheids and mature sclereids in various stages undoubtedly may prove to be a transitory stage in the formation of storage tracheids into mature sclereids. One could see in the hybrid cells the various stages of elimination of characters common to storage tracheids and the manifestation of sclereid characters of thick striated wall leading to a narrow or occluded lumen. However, the mode of transformation and the causes, if there are any, for such transformation remain to be proved ontogenetically. Anyhow we can say that the water storage function diminishes when once the storage tracheids are on their way to sclerosis. This factor should be borne in mind in attributing the functions of water storage or conduction in early stages and the mechanical function of strengthening the cells at the adult stage.

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Summary

The present study describes the salient features of foliar sclereids and their distribution in the mesophyll of 14 species of *Linociera*. Sclereids are the most prominent idioblasts and mostly exhibit diffuse pattern of distribution and rarely do they exhibit apparent terminal relationship with veinlets. Ontogenetic studies have, however, revealed that the initials of the seemingly terminal sclereids arise away from the procambial strands. They are transformed spongy cells in *Linociera insignis* whereas in *L. intermedia* and *L. elliptica* they are palisade cells and the apparent terminal appearance is due to juxtaposition or vigorous development near the procambium. Thus, sclereids should not be designated as terminal merely on the basis of their position in mature leaf unless this has been compared by an ontogenetic study. On the basis of morphological data, sclereids are divided into six compact types *viz.*, spherosclereids, osteosclereids, fusiform sclereids, filiform sclereids, astrosclereids and crystalliferous sclereids. The problem of classifying sclereids is discussed and a classification of foliar sclereids, based on their ontogeny and general morphology, is emphasised. The function of sclereids as mechanical idioblasts is discussed with other possible physiological functions of sclereids.

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STUDIES ON THE PENETRATION OF LIGHT IN THE BAY OF BENGAL PART I—TRANSPARENCY OF THE WATERS ON THE EAST COAST OF INDIA AND ITS SIGNIFICANCE

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Introduction

Absorption of radiation is one of the important physical properties of sea water and the rôle played by the solar radiation in the surface layers of the waters is too well known to need to be explained here. From the biological point of view, it is now recognized that the distribution and intensity of light beneath the surface of the sea is of importance in the photosynthesis of aquatic plants, the formation of vitamin D stored in the liver oils of fish, and the movements of the photosensitive animals. It is now an almost established fact that certain groups of macroplanktonic organisms like Chaetognaths, Copepods and others (Michael 1911; Russel 1926, 1936; Clarke 1933; Harris 1953; and Hardy and Bainbridge 1954) show a well defined vertical positive and negative migration having a direct relationship with the spectral distribution of the light during the different times of the day. Atkins and Poole (1933), Atkins (1932) at Plymouth, Oster and Clarke (1935) and Clarke (1933, 1936) have made extensive observations in the mid-Atlantic and Pacific areas on the phenomenon of light penetration, using specialized photoelectric instruments.

Our knowledge of the transparency of the waters of the Bay of Bengal is almost nil but for a brief reference to it by Prasad (1952). The present paper is a result of the analysis of the data collected during the various oceanographical cruises (Table 2) conducted by the Andhra University during 1952-53. The object of this paper is to give a factual account of the distribution and variation in the transparency of the waters for the visible portion of the light as obtained from Secchi disc readings, an appraisal of their importance in the general distribution of Chaetognaths on this coast and of their significance in the existance of the two water masses on this coast during the Northeast (October–December) and Southwest monsoon (April–September) periods.

Material and Methods

A Secchi disc of the standard size (30 cms. diameter) painted white and carrying a weight of 4 lbs. was lowered on the convenient side of the ship on a rope line graduated in feet. It should be admitted here that Secchi disc readings for measuring the transparency of the waters are liable to be vitiated by errors of the vision of the observer, the wire angle deviation, sun's altitude, weather and sea surface. In the absence of photoelectric instruments, the data presented can only have a general significance and will have to be confirmed by future observations with more sensitive and standard instruments. The positions where

Secchi disc readings were taken are given in Table 2 appended to this paper and are located on the charts 1, 2 and 3.

AREA COVERED

(Charts 1, 2 and 3 and Table 2)

The area in which the Secchi disc readings were taken extends between parallels 13°11.5′ Latitude North (off Madras) and 21°25.0′ Latitude North (near Swatch of No Ground) and the meridians 80° 21.4′ Longitude East and 89° 41.0′ Longitude East. The observations were mostly in the 0–25 mile zone all along the East coast of India. The period covered is from November 1952 to April 1953, as from April onwards till the end of October, the conditions in the Bay are unsettled owing to the onset of the Southwest monsoon winds and cyclones. During this period the small minesweepers of the Indian Navy on which the oceanographical cruises were conducted, are not allowed to cruise in the Bay of Bengal.

SOME GENERAL ASPECTS OF LIGHT PENETRATION AND TRANSPARENCY OF THE WATER

It is recognized that "the measured extinction coefficients (k) will be independent within wide limits of the altitude of the sun" in a given area (Sverdrup 1952). A critical perusal of the appended Table clearly reveals the great difficulty involved in establishing a definable relationship between the Secchi disc readings and the position of the sun in the sky, cloudiness, visibility and sea surface. However, most of the cruises were conducted under ideal conditions of sun and weather.

It is often stated that large phytoplankton outbursts should give low Secchi disc readings, because of the added organisms but the filtered water may be clearer. Many workers (Marshall 1933; Russel and Colman 1934; Petterson 1934*a*) have shown an inverse relationship between the phytoplankton and the visible range of the Secchi disc. In fact Clarke (1946) has indicated the possible relationship between the transparency of the waters measured in terms of Secchi disc readings and the abundance of phytoplankton expressed in Harvey units per m^3 : it was observed that the Secchi disc disappeared at or less than 8 meters whenever the plant pigment exceeded 40,000 Harvey units. In this connection it will be of interest to quote Atkins (1932) who found that at Station E when the phytoplankton outburst was at its height. the water was found to be clearer than ever previously recorded. In a later paper the same author (Atkins *et al.*, 1954) states that such clearing of the water may be due to the sinking of the diatoms and their consumption by animals. "Further the extinction coefficient is not largely affected by scattering when it is determined by measuring the illumination falling on horizontal plates, since light scattered out of the direct beam is compensated for by light scattered in" (Atkins *et al.* 1954).

Clarke and James (1939) have concluded that the large k observed in the sea is mainly due to the presence of filter passing materials found in suspended condition and yellow substances (Kalle 1938). The last named are presumed to be a metabolic production of the phytoplankton of the sea and its origin and distribution are not very well known. This product is considered to be responsible for the large k encountered in the coastal waters and the suspended material for it in the offshore waters.

The works of Jerlov and Koczy (1947) and Jerlov (1950, 1951) have indicated that different water masses may be recognized by their different extinction co-

efficient and particle content. In a recent paper Emery (1954) has found close relationship between the transparency of the waters, as indicated by Secchi disc readings, off Southern California and the supply of detrital and to a lesser extent organic sediment along the mainland and island shores and to organic production in a large area of upwelling near the continental slope.

The foregoing account suggests that the factors responsible for the variation and distribution of the transparency of the waters are mainly due to suspensoids. As to the part played on this coast by the yellow substances nothing is known. The distribution of the suspensoids is largely dependent on water movements which on this coast are greatly influenced by the monsoons.

Currents

The waters of the Bay of Bengal are subject to the influence of two important factors. Firstly, the major rivers of India and Burma empty themselves into the Bay of Bengal. To the East of it the Irrawady, to the North of it the Ganges and the Brahmaputra and on the West the Mahanadi, the Godavari, the Krishna and the Cauvery bring large volumes of silt-laden fresh water especially during the monsoons. Secondly the Northeast and the Southwest monsoons set up, by their influence, four distinct patterns in the movement of the surface waters of the Bay during the various months of the year.

The conditions in the Bay during the months for which the data are available are as follows:

In November-December (Chart 4) the West drift of the Northeast monsoon is fully established all over the Bay with a well defined anticlockwise circulation of the surface waters. As there is a constant Westerly drift from the central portion of the Bay towards the East coast, the turbid deltaic waters from the head of the Bay and the Mahanadi estuary get caught up in this circulation and kept close to the shore and are transported all along the East coast. These waters can with justification be expected to carry appreciable quantities of silt and other suspended matter and are therefore less transparent. In the February-April period there is a reversal in the direction of the water movement and the surface waters of the Bay show a clockwise circulation. A reference to the Chart 4 reveals that the waters along this coast now passing in a Northerly direction appear to come mainly from the region South of the parallel 10° Latitude North and are characterized by higher transparency because of their barren and oceanic nature.

For the purposes of the study of the distribution of the transparency the area covered has been divided into four regions. They are, (A) area opposite Visakhapatnam (Chart 1), (B) area opposite Kakinada coast (Chart 2), (C) the Swatch of No Ground area opposite the mouths of the Ganges and the Brahmaputra (Chart 3) and (D) the general coastline extending from Madras on the South to Swatch of No Ground on the North (Chart 3).

The Extinction Coefficient (k)

The extinction coefficient is a measure of reduction of light intensity on a vertical distance in the sea and is defined by means of a coefficient similar to the absorption coefficient,

$$k = 2.30 \text{ (log } I_z - \log I_{z+1}$$

where I_z and $I_{(z+1)}$ represent the radiation intensities of wave length on horizontal surface at depths z and $(z+1)$ meters. Comparison of the Secchi disc readings with the more accurate measurements by the photoelectric instruments

have indicated that the extinction coefficients of the visible rays can be obtained for any given area by the use of the formula

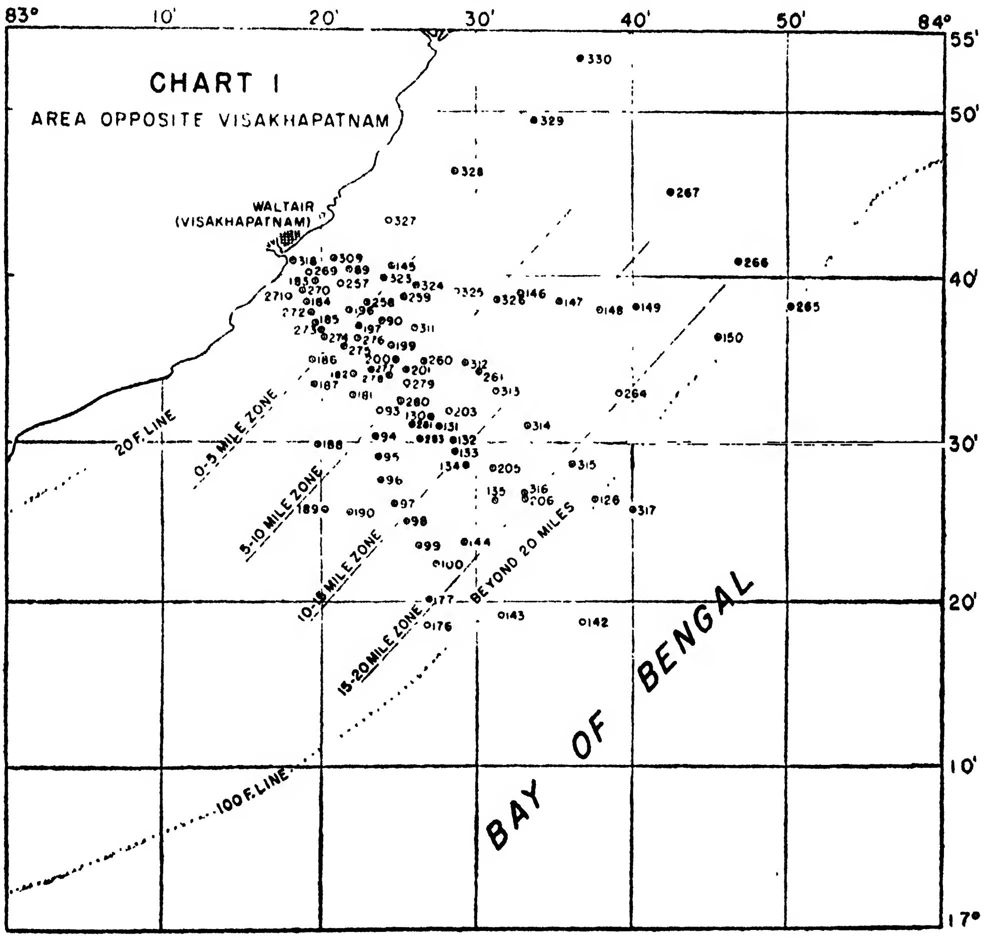
$$k = 1.7/D \text{ in meters} \quad \text{or} \quad k = 5.8/D \text{ in feet}$$

where D is the maximum depth of visibility either in feet or meters, as determined by the Secchi disc readings (Sverdrup 1952). According to this the largest extinction coefficient was obtained at station No. 154 where $k = 1.210$. The lowest values were recorded at stations 131, 214 and 216, the k being 0.041.

A. Area Opposite Visakhapatnam

(Chart I)

Secchi disc readings have been taken from 71 stations off Visakhapatnam and, for convenience of correlation, the stations have been grouped into 5 zones *viz.*, 0–5 miles, 5–10 miles, 15–20 miles and 20 miles and beyond.



0–5 mile zone:

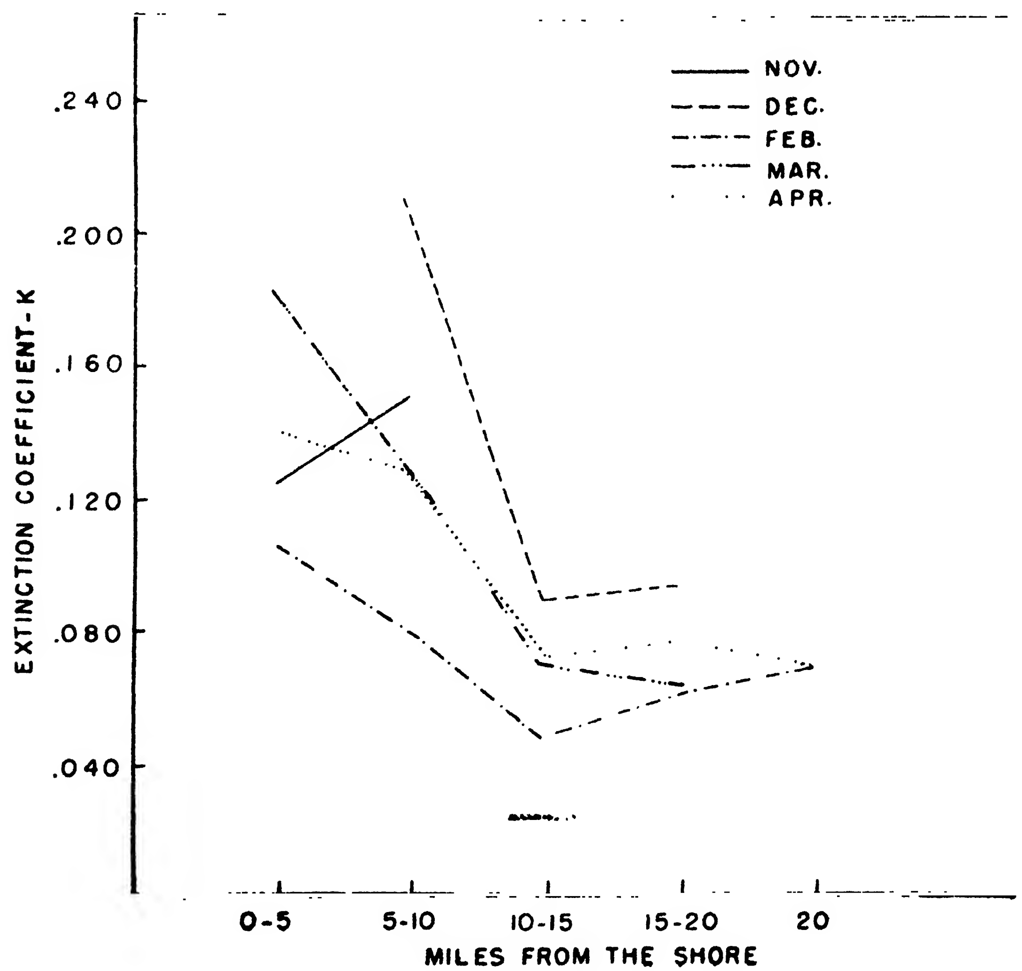
Station numbers are 89, 145, 183, 185, 196, 257, 258, 269, 270, 272, 309, 323 and 327. (Table 2 for details).

It is known that the shore waters are polluted by seepage from the land and is constantly disturbed by the tides and waves. As a result there is a higher concentration of the suspended matter in these agitated waters reducing the transparency and thus accounting for the large k. This may also be due in part to the rich concentration of plankton which in its turn may contribute to the formation of the yellow substances, which have been shown to be high class absorbents of radiation, particularly in the coastal waters (Kalle 1938). Other workers (Ganapati and Rao 1953; Menon 1931) have recorded during the months of October-December and April and June richness of plankton in the inshore waters, thus supporting the above conclusion.

5–10 mile zone:

Station numbers are 90, 93, 182, 187, 199, 200, 201, 275, 276, 277, 278 and 279, 311, 324, 325 (for details refer Table 2).

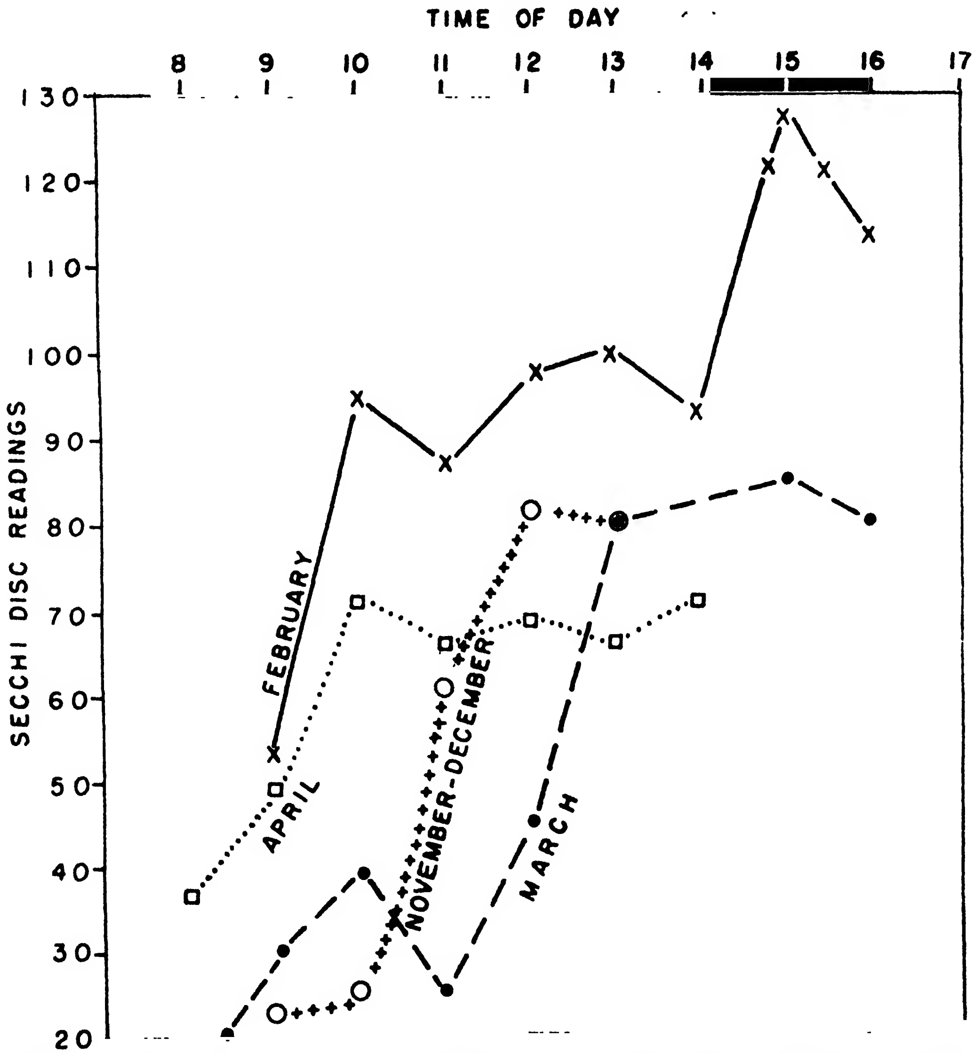
The transparency in this zone is not very different from the previous zone and the indications are that the k value is higher in the November-December period (Table I) with an appreciable decrease in the February-April period.



TEXT-FIG. 1

Through the zones, 10–15 miles (Stations 94 to 97, 130 to 133, 146, 147, 189, 190, 261, 280, 283, 312, 313 and 326), 15–20 miles (Stations 98 to 100, 134, 136, 144, 148, 149, 205, 206, 264 and 316), and 20 miles and beyond (Stations 126, 142, 143, 150, 176 and 315) the waters become clearer as is indicated by their Secchi disc readings. A study of the different mile zones (Text-Fig. 1) reveals that there is more or less a steady and progressive diminution in the turbidity of the water as we pass from zone to zone till we reach the 20 mile limit which appears to lie near the edge of the continental shelf.

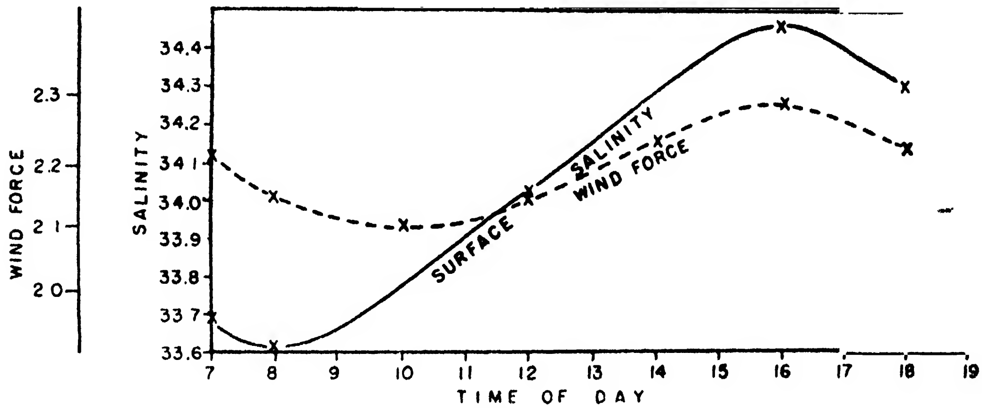
Further, the seasonal variation in the extinction coefficient of the waters off Visakhapatnam reveals certain interesting features. If we consider the differences in the Secchi disc readings at different times of the day in different months of the



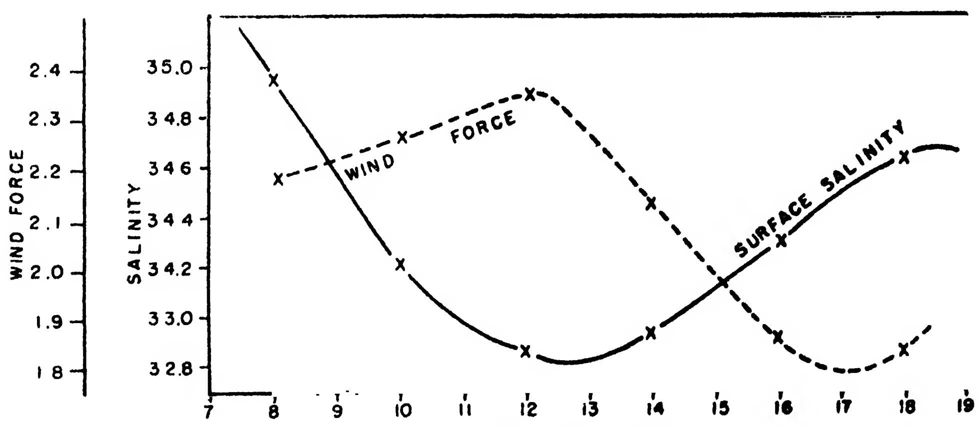
TEXT-FIG. 2. Secchi disc readings at different times of the day in different months.

year, we get the curves shown in Text-Fig. 2. It is clear that the transparency of the water is relatively low in November-December, is highest in February and then diminishes again in March-April and that in all the three months, February, March and April there appears to be a small rise in transparency at 10 : 00 hours

and a major peak at or near 15 : 00 hours. Sewell (1929) has pointed out that the evidence then available seemed to indicate the existence of a double diurnal rise and fall in the salinity of the surface water (Text-Fig. 3), probably brought about by upwelling from some depth below, under the influence of changes in the wind force as a result of changes in barometric pressure. He (*loc. cit.*, p. 339) has also pointed out that "in October the surface salinity shows a positive phase that is characteristic of the wet season of the year.... It appears from January results that the phase has been reversed during the preceding months and during certain



Bay of Bengal salinity and wind force October, 1921 positive phase.

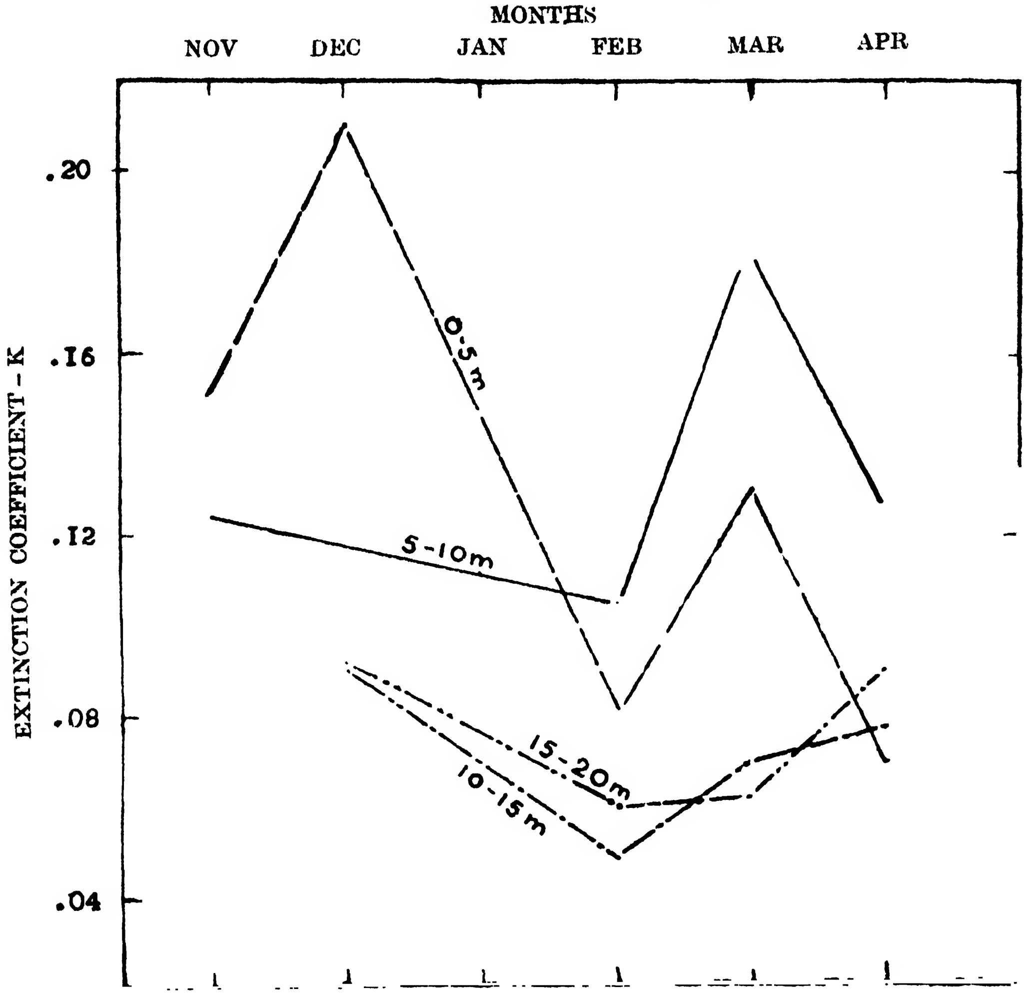


Bay of Bengal salinity and wind force January, 1923 negative phase.

TEXT-FIG. 3. (after Sewell)

years the negative or dry season phase with a high salinity may even persist till January. From February till April the phase is once again positive." The positive phase is caused by upwelling of more saline waters thus raising the salinity of the surface waters that has been reduced by rainfall or influx of river water, while the negative phase is due to upwelling of less saline water into the surface stratum that has had its salinity raised by evaporation. It is of interest to note that the interruption in the rise of the transparency that occurs in February, March and April, coincides as regards time between 11 : 00 and 14 : 00 hours, with the time of minimum salinity when upwelling, if this be its cause, was at its maximum and was bringing into the surface stratum an increased quantity of plankton that had sunk or migrated into the deeper levels at sunrise.

The average of the Secchi disc readings taken in different months of the year in the Bay of Bengal (Prasad 1952) shows a marked rise from the month of August through November and December to a peak in February-April. If the data of the value of k given in Table I is plotted out as above (Text-Fig. 4a) we get the result that



TEXT-FIG. 4(a).

the turbidity is probably high in all five zones in November and rises still further in December to a 1st maximum. It then falls in all zones till February when it reaches a minimum. From this month it again rises sharply in all zones to a 2nd maximum in March in zones 0–5 and 5–10, which is followed by a fall in April, and in zones 10–15, 15–20 and 20 and beyond this rise is continued. If this be compared with the quantities of Phytoplankton and Copepoda in different months as given by Menon (1931) and Ganapati and Murthy (1954), it seems probable that the first marked fall in the transparency and the corresponding rise in turbidity of the water corresponds exactly with the Copepod maximum in December, and the following phase of decreased transparency and increased turbidity occurs in February-March and coincides with the fall in the Copepod population and the beginning of the rise in the Diatom maximum, which continues through March and April. Between these two phases we have an increase in transparency and a fall in turbidity in January-February and this appears to correspond to th

relatively slight fall in the Diatom population, while the Copepod population is still high and may perhaps be due to grazing by the still large Copepod population. In the inshore waters of the 0–5 and 5–10 mile zones the increased turbidity in March is followed in April by a second phase of diminished turbidity and increased transparency, perhaps due to decrease in the Copepod and other animal populations, but in the offshore zones of 10–15 and 15–20 plus zones the turbidity is still further increased and may be correlated with the greatly increased Diatom population and an accompanying increased production of the "yellow substance." The correlation between the density distribution of the Chaetognaths and the Secchi disc readings indicates the very interesting relationship that the Chaetognath maximum corresponds with the occurrence of highly transparent waters as found in February (Text-Fig. 4b). This period, perhaps, represents a stage when Diatoms are being grazed upon by Copepods and other smaller animal plankton and these in turn are devoured by the carnivorous Chaetognaths. Such a kind of predatory cycle would result in the overall decrease of not only the Diatom population, but also of other smaller Zooplankton leaving behind the higher carnivorous community like the Chaetognaths, thus contributing towards the increase in the transparency of the waters in February.

Further, the seasonal variation in the transparency may also be correlated with the prevailing currents. During the two periods, namely, the November-December and February-April, it is seen that while in the former the waters are more turbid (Table I) in the latter months the lowest extinction coefficients are met with, thus indicating greater transparency particularly in the offshore areas. It is now known (Sewell 1929; La Fond 1954; and Ganapati and Murthy 1955) that the full effects of the prevailing currents are realized in the 5–15 mile zone offshore, and there is sufficient justification for considering the waters of the Southerly current flowing past this coast during the months of November-December to be more turbid as already stated elsewhere. The increased transparency of the waters in the February-April period is explained by the fact that the waters which flow past this coast in this period of the year are of oceanic in their origin (*vide* Sewell 1929, Pt. V, text figs. 81, 82, pp. 286–289).

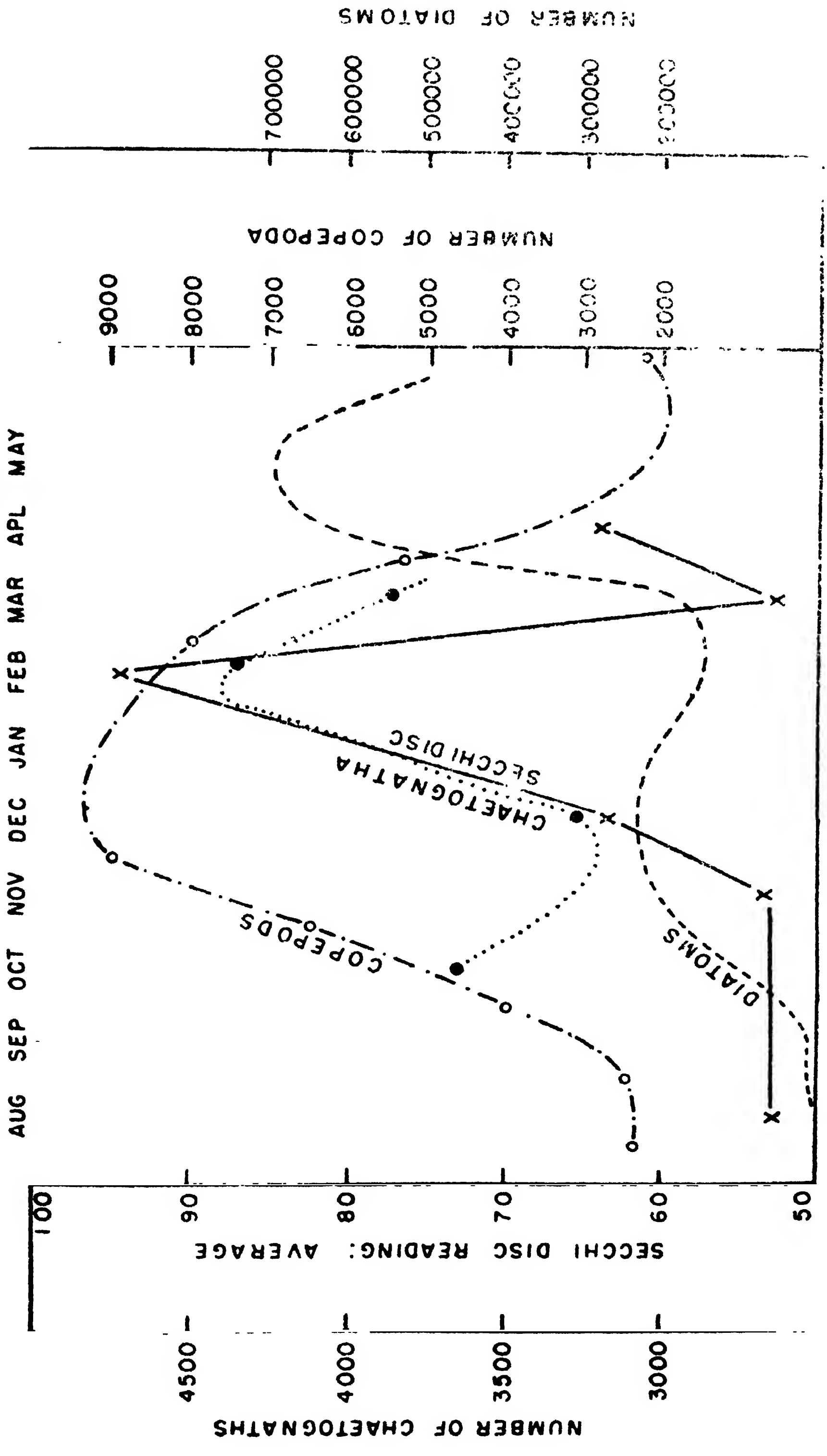
The nature of the bottom in the 3–12 mile zone (approximately) off this coast indicates the presence of a bed of fine clay and silt and this supports the argument (Sewell 1929) that the Southerly current brings along with it silt and other material to be deposited all along this coast. This can be considered as a possible

TABLE I

Seasonal variation in the extinction coefficient in the different mile zones off the Visakhapatnam Coast

Months	Nov.	Dec.	Feb.	Mar.	Apr.
Direction of the current to	South-West	South-West	North-East	North-East	North-East
0— 5 mile zone	0.124(1)	—	0.105(1)	0.182(4)	0.125(10)
5—10 ,,	0.151(1)	0.210(3)	0.081(1)	0.130(5)	0.070(9)
10—15 ,,	—	0.090(4)	0.049(6)	0.070(2)	0.077(5)
15—20 ,,	—	0.092(3)	0.060(6)	0.062(2)	0.090(3)
Beyond 20 miles	—	—	0.068(5)	—	0.124(1)

Number within parenthesis indicates total nubmer of observations.



TEXT-FIG. 4(b). Average Secchi disc reading in different months compared with the Copepod, Diatom and Chaetognath population.

explanation of why the waters of the Southerly current are comparatively less transparent.

General Characteristics of the Two Currents

The turbid current: Otherwise known as the Southerly current of the North-east monsoon.

Period: August to December.

Effective area: 5–15 miles off the coast.

Other features:

1. Low transparency and shallow euphotic zone; range of k 0.090 to 0.210.
2. The Chaetognaths are few in number and variety.
3. Minor planktonic blooms (Ganapati and Murthy 1953).
4. Great fluctuations in salinity (Ganapati and Murthy 1954).
5. The thermocline is at greater depths from the surface (La Fond 1954).

The Transparent current: Otherwise known as the Northerly current of the Southwest monsoon.

Period: Late December to beginning of August.

Effective area: Extensive off the coast.

Other features:

1. High transparency of the waters and deep euphotic zone; range of k 0.049 to 0.182.
2. Very rich populations of Chaetognaths.
3. Major planktonic blooms.
4. Steady conditions in the salinity (Ganapati and Murthy 1954).
5. The thermocline is near the surface (La Fond 1954).

Area B. Area Opposite Kakinada Coast

(Chart 2)

The Secchi disc readings on four sections across the shelf off Kakinada, are available for study. The station numbers are the following:

Section 1. 152, 153, 302, 305 and 307.

Section 2. 154 to 160 and 161.

Section 3. 292, 294, 296, 298 and 299.

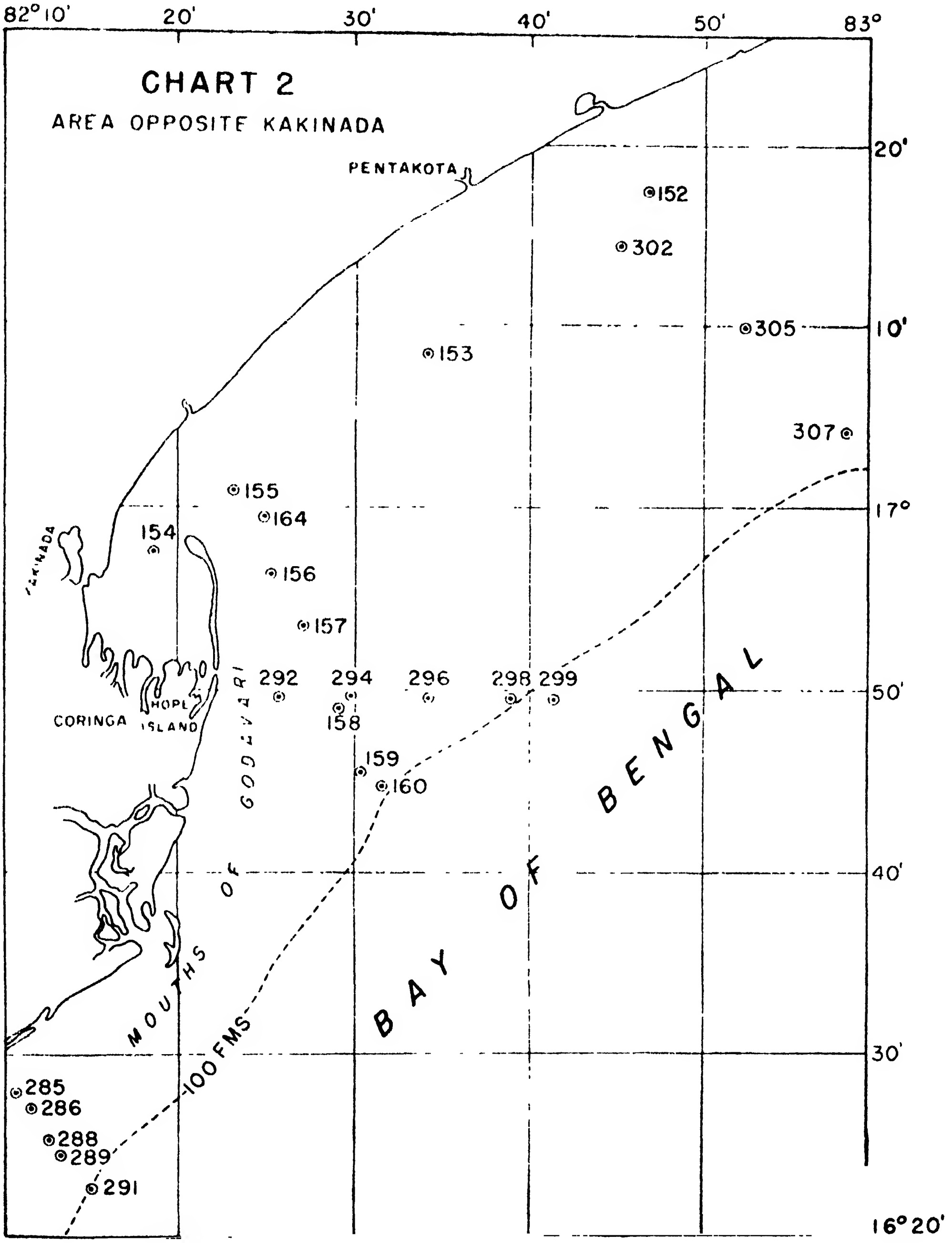
Section 4. 286, 289 and 291.

The coastal waters off Kakinada are estuarine in nature as a result of their proximity to the mouths of the river Godavari. Hence higher turbidity in these waters is to be expected and in fact the highest recorded extinction coefficients like 1.210 (Secchi disc reading 4.8′) and 1.116 (Secchi disc reading 5′) are observed in this region.

The observations on the first section, except for the Station No. 152, are located more than 30 miles away from the Northern mouths of the river Godavari. The waters in this region appear to be beyond the influence of the river, in spite of the fact that there exists a strong Northerly current along this coast during this period of February-April when these observations were taken. It may be stated here that the outflow of waters from the Godavari River is at its maximum in this part of the year, and therefore cannot be expected to contribute much turbidity to these waters, which are comparable to the coastal waters off Visakhapatnam. The value for k ranges between 0.135 to 0.083.

Section 2 was taken roughly parallel to the coast, starting opposite Kakinada town which is situated on the Coringa Bay, into which opens the Coringa branch

of the river Godavari. The waters here are extremely turbid and the largest k observed (1.210) is at Station 154. The observations at all the other stations in this zone indicate an almost uniform but high extinction coefficient. At Station



159 which is about 9 miles from the coast and near the 100 fathom line the k is as high as 1.116, i.e., the Secchi disc disappeared at 5 feet distance from the surface.

The third set of stations (Section 3) starts opposite Hope Island, from about 3½ miles off the coast. The k along this line is large. But the last station situated beyond the edge of the continental shelf (Station No. 299) showed a comparatively

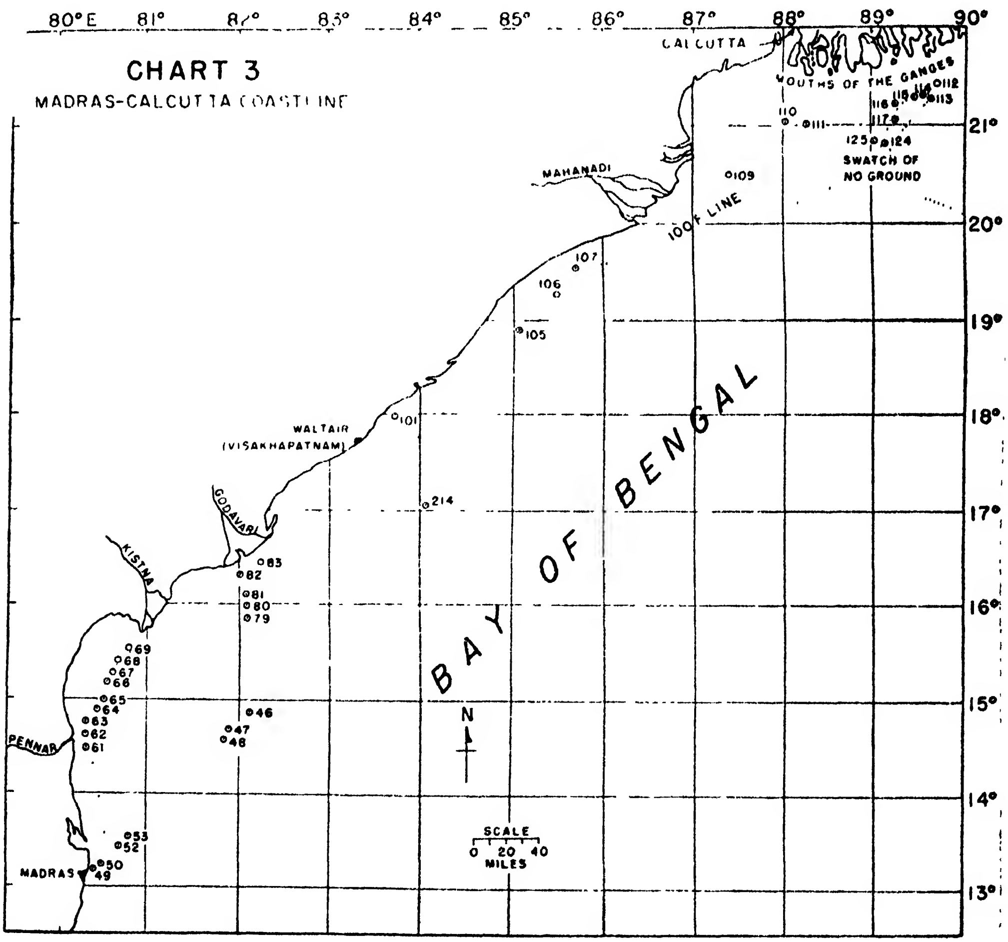
low reading of 0.089. Even the readings along the fourth section which is situated in between the two major mouths of the river Godavari show high values for k ranging between 0.166 to 0.254.

It may be stated in general that the waters off Kakinada—about 20 miles radius with the Hope Island as the centre—are turbid apparently under the influence of the river Godavari.

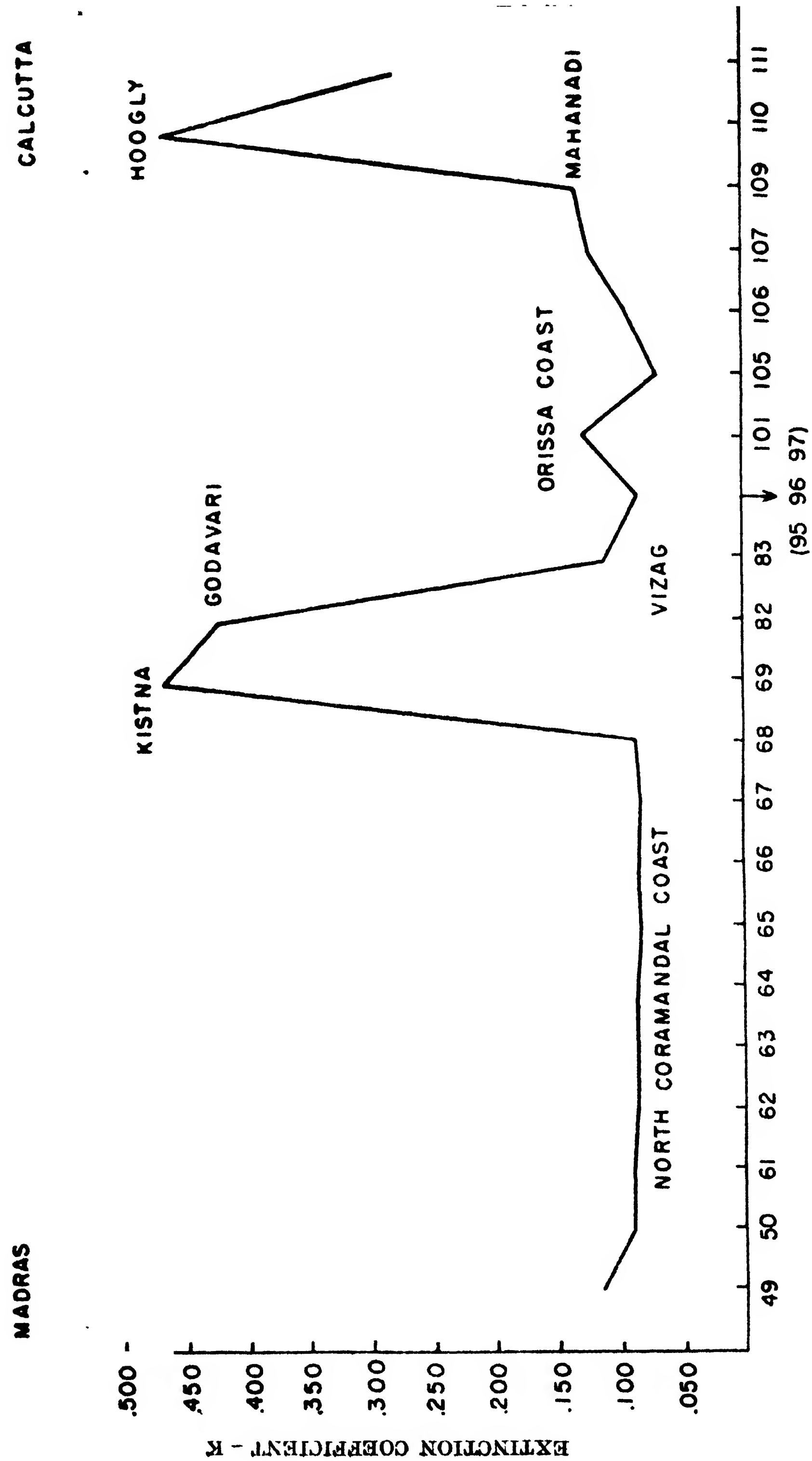
Area C. Swatch of No Ground and Vicinity

(Chart 3)

The station numbers are 110, 117, 124 and 125. The waters over this area in general show relatively high turbidity and the k ranges from 0.079 to 0.485. The Swatch portion of the area appears to be more transparent and the observations taken across a section at stations 113, 114, 115 and 116 speak eloquently in support of this statement. These four observations lie in a line from East to



West across the head of the Swatch of No Ground and the results clearly suggest that there is a well marked fall in the reading on the west side of the Swatch. A similar drop in the reading is indicated at the two stations 124 and 125 which were taken over the West bank of the Swatch further to the South. At stations 110



TEXT-FIG. 5. Station numbers.

and 111 further to the West the Secchi disc readings were much lower, to wit 12 and 20 feet. La Fond (1954) has called attention to the disturbances in the upper stratum of the sea water over the head of the Swatch and he has attributed this to 'rotary currents'. Sewell (1929) has called attention to this region and has indicated that there is a deep current flowing up the Swatch bringing an open sea deposit of Pteropod ooze into the gully. As this deep current flows up the gully, it will by the bottom contours be deflected towards the surface and must finally mingle with the surface water ; such a current of ocean water will possess a low temperature and a high transparency and as it reaches the surface region it may well be split into bands, which as they move surfacewards cause a pushing upwards of the isotherms exactly as La Fond (1954) had indicated in his figure 7. and as the wind at the time when he took his observations was from the Northeast the surface waters will be deflected westwards and thus may well cause a high reading over the Swatch to be rapidly reduced to a much lower reading as the surface water is driven westward to mingle with the highly turbid water of the Gangetic estuary.

Area D. General Coastline (Between 0–20 Miles Mostly)

(Refer Chart No. 3)

It is interesting to note the existence of uniformly transparent waters all along the east coast of India, from near Madras on the South to Swatch of No Ground on the North, during the months of November-December, 1952, when the Secchi disc readings were taken. The station numbers are 16, 50, 52, 53, 61 to 69, 79 to 83, 101, 105 to 107 and 109 to 111. A perusal of the Text-Fig. 5 reveals theexistence of almost similar transparency at most of the stations except those opposite estuaries and this can only mean the presence of an over-riding factor which on this coast is considered to be the surface current flowing in the Southerly direction bringing along with it turbid waters from the Ganges and the Mahanadi estuaries (Sewell 1929).

Acknowledgments

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Summary

1. A total number of 158 Secchi disc readings taken during the various oceanographical cruises conducted by the Andhra University from October 1952 to April 1953, all along the east coast of India, extending from Madras in the South to the Swatch of No Ground in the North, have been analyzed for determining the general transparency of the waters in the area.

2. The highest extinction coefficient of 1.210 at Station 154 near Kakinada and the lowest of 0.041 at Stations 131, 214 and 216 off Visakhapatnam were recorded.

3. It has been found that during the November–December period the waters all along the east coast of India show a uniform transparency apparently under the influence of the prevailing Southerly current.

4. The existence of the two water-masses off Visakhaptnam has been further supported by their different extinction coefficients. Alternate names, the turbid current for the Southerly

current and the transparent current for the Northerly current are suggested and their characteristics are defined.

5. The general transparency of the waters off Visakhaptnam and its significance is described in greater detail.

6. Hydrophotometer studies on the distribution of the turbidity of the waters off the East coast of India by La Fond and Sastry (1957) are found to be complimentary to these findings based on Secchi disc readings.

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TABLE 2

St. No.	Date	Time	Position Lat. N.	Position Long. E.	Depth (feet)	Wind force	Sea	Cloud amount	Visibility	Secchi disc (feet) & K
				Cruise No. 3—I.N.S. Madras—19th November, 1952						
46	20-11-52	14-05	14 50.0	82 04.0	10200	4	4	8	8	75—0.075
47	,,	15-54	14 42.0	81 53.0	,,	4	4	10	8	51—0.114
48	,,	17-04	14 35.0	81 43.0	,,	4	4	10	8	60—0.095
49	23-11-52	09-30	13 11.5	80 21.4	78	4	3	1	8	49—0.119
50	,,	11-10	13 17.0	80 23.3	234	4	3	1	8	63—0.092
52	,,	14-03	13 29.0	80 40.5	3000	4	4	4	9	66—0.088
53	,,	16-23	13 32.0	80 46.5	9000	2	2	2	8	56—0.102
61	24-11-52	06-47	14 30.0	80 17.0	104	2	2	3	9	62—0.094
62	,,	07-45	14 36.0	80 17.5	125	1	2	1	9	63—0.092
63	,,	08-40	14 44.0	80 17.7	170	3	3	3	8	65—0.089
64	,,	10-05	14 50.7	80 21.5	250	3	3	4	8	65—0.089
65	,,	11-30	15 00.0	80 26.0	302	3	3	1	8	67—0.086
66	,,	12-28	15 10.0	80 30.0	350	3	2	6	8	66—0.088
67	,,	13-44	15 18.5	80 35.0	600	2	2	1	8	67—0.086
68	,,	14-58	15 26.0	80 40.0	326	3	3	2	9	64—0.091
69	,,	16-32	15 33.5	80 47.5	162	–	1	3	8	12—0.485
79	25-11-52	07-20	15 50.0	82 03.0	240	1	1	2	–	60—0.095
80	,,	08-35	15 57.0	82 03.0	408	2	2	3	8	45—0.129
81	,,	10-40	16 03.6	82 01.0	607	2	2	4	8	41—0.141
82	,,	13-40	16 15.0	81 57.5	127	1	2	1	9	15—0.385
83	,,	16-15	16 21.0	82 09.0	68	1	2	1	9	52—0.111
				Cruise No. 4—I.N.S. Madras—29th November, 1952						
89	29-11-52	09-05	17 40.5	83 21.7	155	2	3	5	8	23—0.252
90	,,	11-30	17 37.0	83 23.7	180	3	4	3	9	37—0.157
				Cruise No. 5—I.N.S. Madras—5th December, 1952						
91	5-12-52	07-54	17 30.3	83 19.5	131	3	2	–	8	23—0.252
92	,,	08-58	17 35.7	83 22.5	200	2	2	1	8	32—0.181
93	,,	09-50	17 31.7	83 23.3	226	2	2	–	9	23—0.252
94	,,	10-26	17 30.2	83 23.3	240	2	2	–	8	48—0.123
95	,,	10-50	17 28.8	83 23.7	265	3	3	–	8	42—0.138
96	,,	11-20	17 27.5	83 23.8	290	3	3	–	8	74—0.078
97	,,	11-47	17 26.0	83.24.8	321	3	3	0	8	91—0.064
98	,,	12-24	17 24.9	83 25.7	340	3	3	0	8	80—0.0725
99	,,	12-45	17 23.5	83 26.3	420	3	3	1	9	83—0.070
100	,,	13-20	17 22.3	83 27.4	505	3	3	0	8	80—0.072

TABLE 2—(*Contd.*)

St. No.	Date	Time	Position Lat. N.	Position Long. E.	Depth (feet)	Wind force	Sea	Cloud amount	Visibility	Secchi disc (feet) & K
			Cruise No. 6—I.N.S. Madras—9th December 1952							
101	9-12-52	13-35	17 55.5	83 40.5	140	2	2	6	7	43—0.135
105	10-12-52	07-30	18 52.0	85 05.0	600	1	2	5	8	81—0.071
106	,,	12-15	19 13.5	85 29.5	600	1	2	6	8	60—0.095
107	,,	16-40	19 32.0	85 43.0	110	1	1	7	7	46—0.126
109	11-12-52	09-30	20 22.0	87 23.5	175	1	1	0	8	42—0.138
110	12-12-52	13-15	21 02.0	88 01.0	64	2	2	0	8	12—0.485
111	,,	15-30	21 00.5	88 15.5	69	3	1	0	8	20—0.290
112	13-12-52	08-42	21 25.0	89 41.0	64	0	2	0	8	20—0.290
113	,,	10-45	21 18.5	89 33.0	195	0	2	0	8	60—0.095
114	,,	11-35	21 18.5	89 27.0	1000	1	1	0	8	64—0.091
115	,,	13-26	21 18.5	89 27.0	195	1	2	1	8	44—0.132
116	,,	14-50	21 13.0	89 13.0	110	2	2	0	8	35—0.166
117	,,	16-15	21 04.0	89 14.0	600	1	2	0	8	62—0.094
124	14-12-52	06-05	20 50.0	89 05.5	300	3	2	0	7	73—0.079
125	,,	09-00	20 52.0	89 00.0	378	–	–	–	–	68—0.085
			Cruise No. 7—I.N.S. Bengal—17th February, 1953							
126v	18-2-53	11-32	17 26.6	83 37.8	582	1	1	3	9	86—0.067
			Cruise No. 8—I.N.S. Bengal—19th February, 1953							
130	19-2-53	14-43	17 31.3	83 27.0	225	2	–	–	–	127—0.045
131	,,	15-19	17 30.8	83 27.7	240	1	1	5	9	140—0.041
132	,,	16-08	17 30.1	83 28.7	258	1	1	3	9	108—0.056
133	,,	16-24	17 29.3	83 28.6	282	1	1	2	9	114—0.051
134	,,	16-56	17 28.25	83 29.5	319	3	1	4	9	124—0.047
135	,,	17-30	17 26.8	83 31.1	380	3	1	4	7	85—0.068
142	20-2-53	13-42	17 19.0	83 36.8	2600	1	1	5	8	110—0.053
143	,,	14-40	17 19.4	83 31.7	1320	1	1	5	9	106—0.058
144	,,	15-38	17 23.9	83 29.5	390	1	1	8		115—0.050

TABLE 2—(*Contd.*)

St. No.	Date	Time	Position Lat. N.	Position Long. E.	Depth (feet)	Wind force	Sea	Cloud amount	Visibility	Secchi disc (feet) & *K*
			Cruise No. 9—I.N.S. Bengal—24th February, 1953							
145	24-2-53	09-06	17 40.5	83 24.5	156	3	1	2	9	53—0.109
146	,,	10-50	17 39.0	83 32.9	210	3	1	4	9	95—0.061
147	,,	11-44	17 38.1	83 35.3	215	3	1	4	9	104—0.056
148	,,	12-45	17 38.0	83 37.9	223	3	1	5	9	97—0.060
149	,,	13-30	17 38.3	83 40.2	270	3	1	6	9	81—0.071
150	,,	14-30	17 36.5	83 45.5	390	3	1	6	9	76—0.076
			Cruise No. 10—I.N.S. Bengal—25th February, 1953							
152	25-2-53	13-49	17 17.5	82 47.0	138	3	2	4	8	70—0.083
153	,,	16-00	17 08.5	82 34.5	108	4	3	3	8	60—0.095
154	26-2-53	07-15	16 58.4	82 18.7	24	1	1	3	8	4.8—1.210
155	,,	09-20	17 01.5	82 23.4	60	1	1	1	8	10—0.580
156	,,	10-29	16 57.0	82 25.6	102	1	1	1	8	35—0.166
157	,,	11-16	16 53.3	82 27.3	104	2	1	1	8	12—0.485
158	,,	12-05	16 48.5	82 29.3	222	2	1	1	8	25—0.233
159	,,	12-54	16 45.2	82 30.5	324	2	1	1	8	5—1.116
160	,,	13-51	16 43.4	82 31.8	600	2	1	1	8	10—0.580
164	,,	17-30	16 59.3	82 25.0	84	3	2	1	9	50—0.111
176	27-2-53	06-30	17 15.5	83 27.0	480	2	2	3	9	30—0.194
177	,,	07-09	17 20.6	83 27.0	420	2	2	3	9	55—0.106
179	,,	08-49	17 27.6	83 25.5	270	3	2	4	9	105—0.057
180	,,	09-37	17 29.0	83 24.0	222	2	2	5	9	90—0.064
181	,,	10-23	17 32.5	83 22.0	204	3	2	3	9	50—0.111
182	,,	11-40	17 34.0	83 22.1	183	3	2	2	9	70—0.083
			Cruise No. 11—I.N.S. Bengal—2nd March, 1953							
183	2-3-53	08-45	17 39.2	83 19.5	131	3	2	1	9	13—0.446
184	,,	09-32	17 33.2	83 19.2	150	3	2	1	9	24—0.243
185	,,	10-07	17 37.0	83 19.7	165	3	2	1	9	40—0.145
186	,,	10-45	17 34.8	83 19.4	168	3	2	1	9	40—0.145
187	,,	11-42	17 33.3	83 19.7	186	3	2	1	9	25—0.235
188	,,	12-52	17 29.3	83 20.0	228	3	2	1	9	30—0.194
189	,,	13-41	17 25.7	83 20.3	240	3	2	1	9	40—0.145
190	,,	14-35	17 25.5	83 22.0	280	3	2	1	9	120—0.048

TABLE 2—(*Contd.*)

St. No.	Date	Time	Position Lat. N.	Position Long. E.	Depth (feet)	Wind force	Sea	Cloud amount	Visibility	Secchi disc (feet) & K
			Cruise No. 12—I.N.S. Bengal—4th March, 1953							
196	4-3-53	09-40	17 37.7	83 22.0	171	1	2	1	9	30—0.194
197	,,	11-00	17 35.9	83 22.5	174	1	2	2	9	30—0.194
199	,,	11-37	17 35.4	83 24.5	192	1	2	2	9	25—0.235
200	,,	12-14	17 34.9	83 24.5	182	1	2	2	9	40—0.145
201	,,	12-39	17 34.3	83 25.5	198	3	1	3	9	50—0.111
203	,,	14-43	17 31.6	83 28.0	234	3	2	1	9	95—0.061
205	,,	15-45	17 28.5	83 30.2	206	3	2	0	9	100—0.058
206	,,	16-32	17 26.6	83 31.1	366	3	2	0	9	80—0.072
214	5-3-53	07-59	17 10.3	84 03.6	8400	3	2	3	9	140—0.041
215	,,	09-03	17 51.0	84 08.7	9000	3	2	2	9	105—0.057
216	,,	13-02	16 59.8	84 14.6	9600	–	2	3	9	140—0.041
217	,,	14-30	16 54.6	84 19.9	,,	–	1	0	9	100—0.058
			Cruise No. 14—I.N.S. Rajaputana—19th April, 1953							
257	10-4-53	09-04	17 39.8	83 21.0	114	2	2	2	9	29—0.200
258	,,	09-50	17 39.4	83 23.3	132	2	2	2	9	38—0.153
259	,,	10-17	17 38.8	83 25.3	144	3	2	1	9	67—0.086
260	,,	10-59	17 38.1	83 27.3	162	3	2	1	9	78—0.074
261	,,	11-58	17 37.2	83 30.1	168	3	2	1	9	90—0.064
262	,,	12-45	17 36.2	83 38.2	210	3	2	1	9	80—0.072
263	,,	13-30	17 35.1	83 36.2	264	3	2	0	9	85—0.068
264	,,	14-22	17 33.5	83 39.2	288	4	2	0	9	79—0.073
			Cruise No. 15—I.N.S. Rajaputana—13th April, 1953							
265	13-4-53	14-00	17 38.8	83 56.0	636	4	4	1	9	60—0.095
266	,,	14-58	17 41.0	83 46.7	288	3	4	1	9	70—0.083
267	,,	15-37	17 45.0	83 42.5	216	4	4	3	9	70—0.083

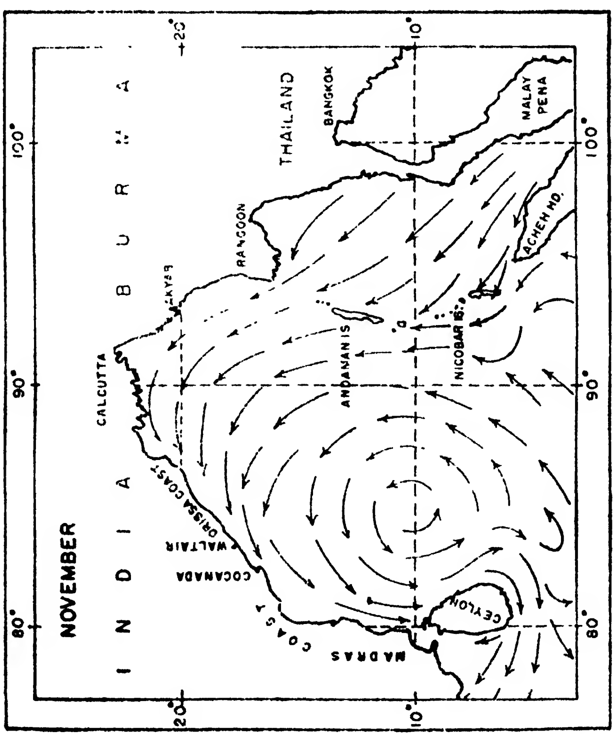
TABLE 2—(*Contd.*)

St. No.	Date	Time	Position Lat. N.	Position Long. E.	Depth (feet)	Wind force	Sea	Cloud amount	Visibility	Secchi disc (feet) & K
			Cruise No. 16—I.N.S. Rajaputana—17th April, 1953							
269	17-4-53	08-42	17 40.0	83 19.4	93	2	1	2	9	12—0.485
270	,,	10-15	17 39.7	83 17.9	66	2	1	0	9	14—0.415
271	,,	10-58	17 39.5	83 18.6	114	3	1	2	9	30—0.194
272	,,	11-14	17 38.7	83 19.4	144	3	1	2	9	42—0.138
273	,,	11-33	17 37.8	83 20.8	156	3	1	2	9	54—0.103
274	,,	12-07	17 37.0	83 20.8	158	3	1	2	9	80—0.072
275	,,	12-25	17 36.5	83 21.3	174	3	1	2	9	100—0.058
276	,,	12-43	17 35.6	83 22.0	180	3	1	2	9	90—0.064
277	,,	13-00	17 34.8	83 23.0	180	3	1	2	9	75—0.075
278	,,	13-15	17 34.5	83 33.5	192	3	1	2	9	80—0.072
279	,,	13-45	17 33.5	83 24.5	204	3	1	0	9	85—0.068
280	,,	14-03	17 32.8	83 24.8	214	3	1	0	9	85—0.068
281	,,	14-19	17 31.8	83 25.8	216	3	3	1	9	80—0.072
283	,,	15-06	17 30.0	83 27.0	222	3	2	2	9	70—0.083
284	,,	15-21	17 29.2	83 27.6	228	3	2	2	9	75—0.075
			Cruise No. 17—I.N.S. Rajaputana—20th April, 1953							
286	21-4-53	07-25	16 26.7	82 11.6	42	1	1	3	9	35—0.166
289	,,	08-17	16 23.8	82 13.2	300	1	1	4	9	22—0.254
291	,,	08-54	16 22.0	82 15.0	624	1	1	4	9	25—0.233
292	,,	13-02	16 49.3	82 25.6	108	1–2	1	3	9	45—0.129
294	,,	13-34	16 49.9	82 30.0	210	1–2	1	4	9	64—0.091
296	,,	14-13	16 49.5	82 34.2	318	1–2	1	5	9	52—0.111
298	,,	15-07	16 49.3	82 39.0	444	1–2	2	6	9	28—0.215
299	,,	15-38	16 49.3	82 41.0	528	1–2	2	6	9	62—0.094
302	22-4-53	09-12	17 14.3	82 45.4	138	0	1	1	9	43—0.135
305	,,	10-21	17 10.0	82 53.0	216	0	1	1	9	58—0.100
307	,,	11-27	17 05.0	82 58.2	468	0	1	2	9	70—0.083
309	24-4-53	08-57	17 41.5	83 21.4	99	1	1	1	9	60—0.095
310	,,	09-38	17 39.7	83 23.9	162	1	1	1	9	60—0.095
311	,,	10-04	17 37.4	83 26.4	180	1	1	1	9	60—0.095
312	,,	10-50	17 35.8	83 29.9	198	1	1	1	9	60—0.095
313	,,	11-33	17 33.6	83 31.4	248	2	1	1	9	55—0.106
314	,,	12-02	17 31.6	83 33.9	288	2	1	1	9	50—0.111
315	,,	12-32	17 29.5	83 36.4	348	2	1	1	9	45—0.129
316	,,	13-14	17 27.5	83 33.9	624	2	1	1	9	55—0.106

TABLE 2—(*Contd.*)

St. No.	Date	Time	Position		Depth (feet)	Wind Force	Sea	Cloud amount	Visibility	Secchi disc (feet) & K
			Lat. N.	Long. E.						
Cruise No. 19—I.N.S. Rajaputana—27th April, 1953										
319	27-4-53	08-33	17 40.8	83 19.9	93	1	1	1	9	55—0.106
320	,,	08-45	17 40.6	83 21.1	114	1	1	1	9	58—0.100
321	,,	09-10	17 40.3	83 22.2	144	1	1	1	9	60—0.095
322	,,	09-31	17 40.1	83 23.2	150	1	1	1	9	65—0.089
323	,,	09-47	17 39.8	83 24.4	162	1	1	1	9	80—0.072
324	,,	10-11	17 39.2	83 26.4	171	1	1	1	9	80—0.072
325	,,	10-34	17 38.8	83 28.5	183	1	1	1	9	82—0.071
326	,,	10-53	17 38.2	83 31.5	192	1	1	1	9	92—0.063
327	,,	12-28	17 43.6	83 24.5	120	1	1	1	9	60—0.095
329	,,	13-55	17 49.5	83 33.3	126	1	1	1	9	78—0.074
330	,,	14-42	17 53.0	83 37.5	126	1	1	1	9	67—0.056
331	,,	15-29	17 56.0	83 41.8	126	1	1	1	9	82—0.071
332	,,	16-13	17 58.8	83 46.0	120	1	1	1	9	52—0.110

CHART 4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INDIA
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THAILAND
CALCUTTA
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WALTAIR
COCANADA
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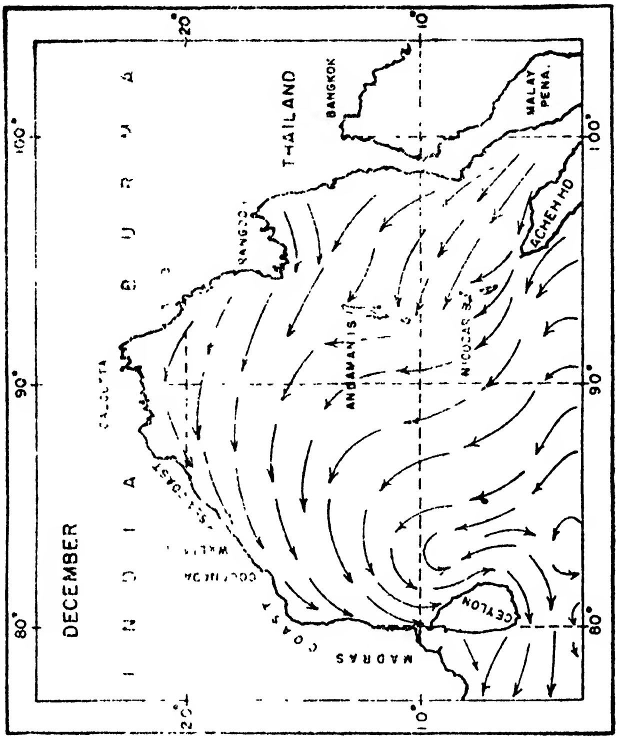
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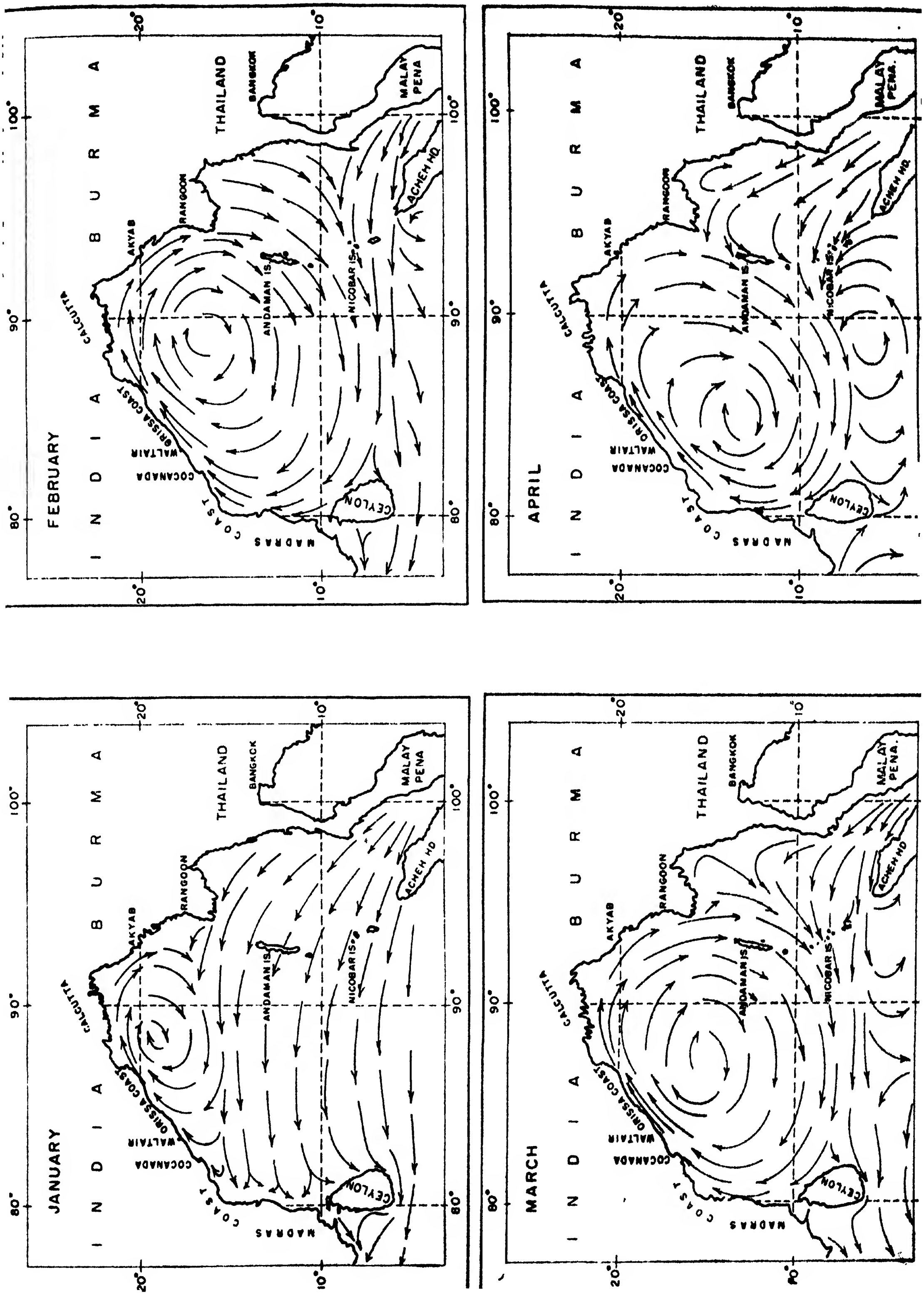
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ORISSA COAST
WALTAIR
COCANADA
MADRAS COAST
CEYLON
ANDAMAN IS.
NICOBAR IS.
MALAY PENA
ACHEH HD
APRIL
INDIA
BURMA
THAILAND
BANGKOK
RANGOON
AKYAB
CALCUTTA
ORISSA COAST
WALTAIR
COCANADA
MADRAS COAST
CEYLON
ANDAMAN IS.
NICOBAR IS.
MALAY PENA.
ACHEH HD
JANUARY
INDIA
BURMA
THAILAND
BANGKCK
RANGOON
AKYAB
CALCUTTA
ORISSA COAST
WALTAIR
COCANADA
MADRAS COAST
CEYLON
ANDAMAN IS.
NICOBAR IS.
MALAY PENA
ACHEH HD
MARCH
INDIA
BURMA
THAILAND
BANGKOK
RANGOON
AKYAB
CALCUTTA
ORISSA COAST
WALTAIR
COCANADA
MADRAS COAST
CEYLON
ANDAMAN IS.
NICOBAR IS.
MALAY PENA.
ACHEH HD
80°
90°
100°
10°
20°

Explanation of Charts

Chart 1. Map showing station locations off Visakhaptnam. The zonal lines are drawn approximately parallel to the coast.

Chart 2. Map showing station locations off Kakinada coast.

Chart 3. Map showing station locations all along the East coast of India, extending from Madras on the South to the Swatch of No Ground on the North.

Chart 4. Maps showing the pattern of the surface currents in the Bay of Bengal during the different months of the year (from Admiralty Chart).

Tables and figures are self explanatory.

Wind force : Indicated by a number given in the Beaufort scale.

Cloud amount :

Code	*Amount of clouds.*
0	No clouds
1	Less than 1/10 or 1/10 of the sky
2	,, 2/10 or 3/10 ,,
3	,, 4/10
4	,, 5/10
5	,, 6/10
6	,, 7/10 or 8/10 ,,
7	,, 9/10
8	,, 10/10
9	Sky obscure.

Visibility:

Code	*Description*
0	50 yards—visibility
1	200 yards—thick fog
2	400 yards—moderate fog
3	1000 yards—fog
4	1 mile —thin fog or mist
5	2 miles—visibility poor
6	5 miles—visibility moderate
7	10 miles—visibility good
8	30 miles—visibility very good
9	over 30 miles—visibility excellent

Sea surface :

Code	*Description*
0	Flat—calm
1	crest of the wave less than one foot
2	,, 1– 3 ft.
3	,, 3– 5 ft.
4	,, 5– 8 ft.
5	,, 8–12 ft.
6	,, 12–20 ft.
7	,, 20–40 ft.
8	,, 40 ft. or over
9	Very rough and confused.

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